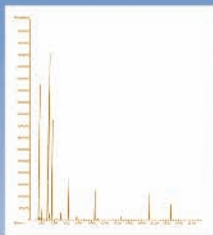


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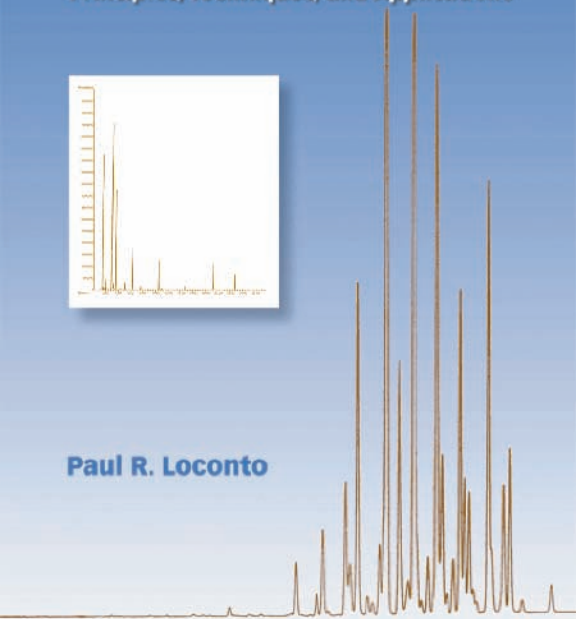
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Trace Environmental Quantitative Analysis

Principles, Techniques, and Applications



Paul R. Loconto



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To Rose Rita (Chivallatti) Loconto (1911–1987)

*She made the bold and insightful suggestion to her academically struggling
high school son that he ought to consider going to college.*

Preface

In the past 20 years, the practice of trace environmental quantitative analysis (TEQA) has exploded! Academic, industrial, and governmental laboratories all use techniques of sample preparation, instrumental analysis, data reduction, and statistical evaluation designed to furnish clients with analytical data from diverse sample matrices such as ground water, surface water, waste water, sludge, sediment, soil, and air. The private environmental testing laboratory industry that expanded during the 1980s and contracted during the 1990s is continually being transformed as new techniques begin to replace older ones. Analytical methods published by the United States Environmental Protection Agency have undergone several revisions during this time period.

All of this innovation, combined with a rapid rate of change fueled by advances in sample preparation, analytical instrumentation, and computer technology, created the need to pose several fundamental questions:

What is a current state-of-the-art environmental analytical laboratory capable of testing, and why, and how?

How has analytical instrumentation evolved in both practice and theory as ever-more precise environmental analyses became desirable, necessary, and achievable?

- How should one teach the principles and practice of TEQA in an academic setting that may have limited resources and facilities?
- What type of environmental chemical analyses would make interesting and instructional student exercises?
- Can a book be written that sustains the interest of the reader while providing the necessary underlying principles and practices of TEQA?
- Can a book, written by a chemist for practicing chemists and for non-chemists such as environmental engineers, extract the essential concepts of TEQA from the nonessential?

This book attempts to answer these questions and is a direct outgrowth of my more than 20 years of experience working in industrial, consulting, and academic analytical laboratories dedicated to TEQA. Few books, if any, follow the approach introduced here. This one places “first things first”! The interpretation of analytical data is, in my opinion, *the* most important task of environmental analytical chemists today. This topic constitutes Chapter 2. Understanding the content of this chapter greatly helps to answer the following question: *What must I do to be able to defend an analytical result that my laboratory releases that pertains directly to environmental contamination?*

The book begins with data reduction and statistical treatment of the data, then focuses on the increasingly important topic of sample preparation in Chapter 3. It continues with a discussion of the most important determinative techniques of analytical instrumentation in Chapter 4 and finishes with a series of detailed experiments in TEQA for students in Chapter 5. Supportive information is found in the appendices.

The book’s content clearly reflects the interest, and therefore the bias, of the author. The separation sciences are given far more coverage than the atomic and molecular spectroscopic sciences. The separation sciences have simply been of greater interest to me than other topics. The effective laboratory manager in the field of TEQA, however, must be equipped to handle any and all requests for analytical services regardless of his prior specialty. This is particularly true in the multi-instrument, environmental testing labs and reinforces the cliché that “variety is the spice of life”.

A moderate amount of mathematical rigor has been introduced because I believe that contemporary texts on the broader subject of analytical chemistry are becoming increasingly watered down. For example, few books show how the least squares regression is derived from first principles. Few, if any, texts elaborate on the physical chemistry that underlies many of the concepts found in TEQA. This book attempts to provide a fresh alternative to such a trend. Several topics have been omitted entirely; these

include a discussion of electronics that provide the interface between instrumentation and the computer, a more advanced discussion of chemometrics, and a discussion of chemical derivatization for gas and liquid chromatography. Physical and aggregate properties for water quality testing such as those found in the 2000 series of *Standard Methods* are likewise not discussed. This omission does not suggest that they are unimportant.

Who should read this book? Students who anticipate performing TEQA will find the content very beneficial. This includes students at universities and four-year colleges that offer an analytical chemistry course with an environmental emphasis. As we have found at Michigan State University, there is a real need to teach incoming graduate students the principles encompassed in this book. The book also serves the practicing analytical chemist who works in an environmental testing laboratory or equivalent.

Who else should read this book? Chemists who would like a fresh approach to TEQA will find it here. Environmental engineers who are engaged in hazardous waste site remediation will find helpful information to clarify practices, procedures, and definitions. These professionals are likely to send samples to environmental testing labs and it is helpful for them to better understand the advantages and limitations of TEQA by better understanding its principles and practices.

I quite innocently entered the realm of TEQA during the early 1980s, in what can be termed the heyday of environmental testing when entrepreneurship was vibrant as brought about by Superfund authorization. I was a thriving community college professor who became involved with a startup company that was attempting to seek state certification for trihalomethanes in drinking water. One thing led to another and before I knew it, I became a student of TEQA, attempting to find a better way to prepare samples than I was reading of in EPA methods, *Standard Methods*, ASTM publications, and so forth.

A few remarks about style are necessary. Not all the mathematical equations given in the book are labeled with a number, only the ones referred to later in the text. Each new section begins with a question instead of a bland header. I have also used both inserted tables and figures as well as the more conventional separated tables and figures. Some graphs are sketches that I drew to illustrate a principle, while others are precisely drawn from real experimental data. I have tried to make the topic of TEQA readable and interesting. I have also attempted to make it something beyond a handbook with a collection of methods and facts, I hope the reader will agree!

The Great Lakes and Mid-Atlantic Hazardous Substance Research Center and the NIEHS Basic Superfund Research Center, both at

Michigan State University (MSU), supported me while this book was being written. Patricia P. Miller, Editor, MSU Extension, reviewed and edited the first drafts. My wife, Priscilla, as she has done for other gargantuan projects, graciously accepted the loss of family time that writing this book demanded. Her continuous and unfailing support for my endeavours is sincerely appreciated.

Paul R. Loconto

Contents

<i>Preface</i>	v
1. Introduction to Trace Environmental Quantitative Analysis (TEQA)	1
2. Calibration, Verification, Statistical Treatment of Analytical Data, Detection Limits, and Quality Assurance / Quality Control	25
3. Sample Preparation Techniques to Isolate and Recover Organics and Inorganics	69
4. Determinative Techniques to Measure Organics and Inorganics	255
5. Specific Laboratory Experiments	471
<i>Appendix A: Glossary</i>	585
<i>Appendix B: QA/QC Illustrated</i>	603
<i>Appendix C: TEQA Applied to Drinking Water</i>	613
	ix

<i>Appendix D: Instrument Designs</i>	623
<i>Appendix E: Useful Internet Links for Environmental Analytical Chemists</i>	629
<i>Index</i>	633

1

Introduction to Trace Environmental Quantitative Analysis (TEQA)

The latter half of the twentieth century has taken the United States and other developed nations from a postindustrial era to a new age that some people refer to as a period that is rich in information technology. We enter the new millenium as a nation in transition, and people want to know to what extent chemical substances have contaminated the environment. In the United States and other postindustrial nations, manufacturing and related human activities that were so dominant in both the nineteenth and twentieth centuries appear to have left their mark on the environment. This book provides the tools necessary to begin to answer the contamination questions by introducing the reader to that part of the science of contemporary analytical chemistry that is devoted to environmental monitoring, testing, and interpretation. All of these aspects are conveniently summarized in the term Trace Environmental Quantitative Analysis (TEQA).

1. WHAT KIND OF CHEMISTRY IS THIS?

The academic discipline of environmental chemistry is a relatively recent development. Environmental chemistry can be defined as a systematic study of the nature of matter that exists in the air, water, soil, and biomass. This definition could be extended to the plant and animal domains where

chemicals from the environment are likely to be found. This discipline which developed in the late 1960s requires the knowledge of the traditional branches of organic, inorganic, physical, and analytical chemistry. Environmental chemistry is linked to biotechnology and also to chemical, environmental, and agricultural engineering practices.

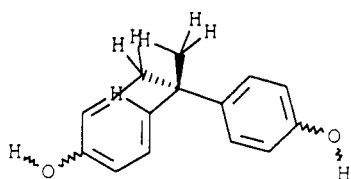
Environmental analytical chemistry can be further defined as a systematic study that seeks to answer two fundamental questions: *What and how* much matter exists in the air, water, soil, and biomass? This definition could also be extended to the plant and animal domains as just discussed. This discipline that developed in the 1970s and was spearheaded by the first Earth Day in 1970 and the establishment of the United States Environmental Protection Agency (EPA) requires a knowledge of traditional quantitative analysis, contemporary instrumental analysis, and selected topics such as statistics, electronics, computer software, and experimental skill. Environmental analytical chemistry represents *the* fundamental measurement science to biotechnology and to chemical, environmental and agricultural engineering practices. That portion of environmental, analytical chemistry devoted to rigorously quantifying the extent to which chemical substances have contaminated the air, water, soil, and biomass is the subject of this book.

In its broadest sense, environmental chemistry might be considered to include the chemistry of everything outside of the synthetic chemist's flask! The moment that a chemical substance is released to the environment, its physico-chemical properties may have an enormous impact on ecological systems, including humans. Researchers have identified 51 synthetic chemicals that disrupt the endocrine system. Hormone disrupters include some of the 209 polychlorinated biphenyls (PCBs), and some of the 75 dioxins and 135 furans that have a myriad of documented effects (1). The latter half of the twentieth century has witnessed more synthetic chemical production than any other period in world history. Between 1940 and 1982, the production of synthetic chemicals increased about 350 times. Billions of pounds of synthetic materials were released into the environment during this period. United States production of carbon-based synthetic chemicals topped 435 billion pounds in 1992 or 1600 pounds per capita (2).

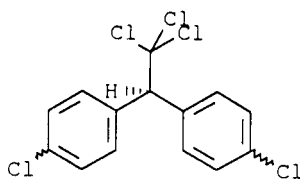
The concept of environmental contaminants as estrogenic "mimics" serves to bring attention to the relationship between chemicals and ecological disruption. The structural similarity between DDT and diethylstilbestrol is striking. The former chemical substance was released into the environment decades ago, whereas the latter was synthesized and marketed to pregnant women during the 1950s and then used as a growth promoter in livestock until it was banned by the Food and Drug Administration (FDA) in 1979 (3).

At levels typically found in the environment, hormone-disrupting chemicals do not kill cells nor do they attack DNA. Their target is hormones, the chemical messengers that move about constantly within the body's communication. They mug the messengers or impersonate them. They jam signals. They scramble messages. They sow disinformation. They wreak all manner of havoc. Because messages orchestrate many critical aspects of development, from sexual differentiation to brain organization, hormone-disrupting chemicals pose a particular hazard before birth and early in life. (4)

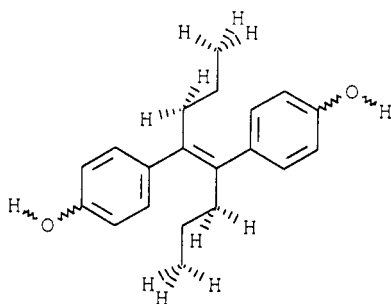
A more recent controversy has arisen around the apparent leaching of bisphenol A from various sources of plastics that are in widespread use among consumers. A dialog appeared recently on this issue and this difference of opinion serves to point out the necessity of measuring bisphenol A in various plastic wraps, containers, and so forth, particularly plastic materials that come in contact with food and beverages (5). These concerns point out the need to find ways to measure this chemical in plastic matrices. How



Bisphenol A



p,p'-DDT



Diethylstilbestrol

FIGURE 1.1 Molecular structures of endocrine disruptors.

would one go about developing such an analytical method? Three-dimensionally accurate molecular structures of bisphenol A, *p,p'*-DDT and diethylstilbestrol are shown in Figure 1.1. All three molecular structures contain two para-substituted phenyl groups that join to a central carbon atom. Is structural similarity related to toxicity?

Recently, the EPA has released its plan for testing 15,000 chemicals for their potential to disrupt hormone systems in humans and wildlife. These chemicals were chosen because they are produced in volumes greater than 10,000 pounds per year (6).

One usually hears about environmental catastrophes through the vast resources of the mass media (i.e., radio, television, newspapers, popular magazines, newsletters from special interest organizations, etc.). The mass media usually assigns a name to the disaster that also includes a geographic connotation. Examples include the Valdez Oil Spill in Alaska, Love Canal in New York, Sevesco, Italy, and Times Beach, Missouri. What is not so newsworthy, yet may have as profound an impact on the environment, is the ever so “subtle” pollution of the environment day in and day out. Both catastrophic pollution and subtle pollution require the techniques of TEQA to obtain data that enable society to continuously monitor the environment to ensure minimal ecological and toxicological disruption. It is the combination of sophisticated analytical instruments (Chapter 4), sample preparation schemes (Chapter 3), mathematical treatment of analytical data (Chapter 2), and detailed practical procedures (Chapter 5) that enables a student or practicing analyst to effectively conduct TEQA.

This book provides insights and tools that enable an individual who either works in an environmental testing lab or who anticipates having a career in the environmental science or engineering field to make a contribution. Individuals are thus empowered and can then begin to deal with the problems of monitoring and sometimes finding the extent to which chemicals have contaminated the environment.

2. WHO NEEDS ENVIRONMENTAL TESTING?

It is too easy to respond and to answer this question by stating “everyone”! The industrial sector of the U.S. economy is responsible for the majority of chemical contamination released to the environment. Since the early 1970s, industry has been under state and federal regulatory pressures not to exceed certain maximum contaminant levels (MCLs) for a variety of so-called priority pollutant organic and inorganic chemical substances. However, one of the more poignant examples of “small-time” pollution is that of dry cleaning establishments located in various shopping plazas throughout the United States. These small businesses would follow the practice of dump-

ing their dry cleaning fluid into their septic systems. It was not unusual, particularly during the 1980s, for labs to analyze drinking water samples drawn from an aquifer that served the shopping plaza and find parts per billion (ppb) concentration levels of chlorinated volatile organics such as perchloroethylene (PCE).

The necessary sample preparation needed to modify a sample taken from an aquifer that is expected to contain PCE so as to enable the sample to become compatible with the appropriate analytical instrument will be described in Chapter 3. The identification and quantitative determination of priority pollutants like PCE in drinking water requires sophisticated analytical instrumentation. These so-called “determinative techniques” will be described in Chapter 4. A laboratory exercise that might introduce a student to the techniques involved in sample preparation and in instrumental analysis to quantitatively determine the presence or absence of a chlorinated volatile organic like PCE will be described in Chapter 5.

3. WHO REQUIRES INDUSTRY TO PERFORM TEQA?

Demand for trace environmental analysis is largely regulatory driven, with the exception of the research done in methods development by both the private sector as well as by federal, state, and academic labs. The major motivation for a company to conduct TEQA is to demonstrate that its plant's effluent falls to within the MCLs for the kinds of chemical contaminants that are released. There exists a myriad of laws that govern discharges and these laws also specify MCLs for targeted chemical contaminants. The following outline is a brief overview of the regulations and it incorporates the abbreviations used by practitioners in this broad category of environmental compliance and monitoring (7).

4. HOW DOES ONE MAKE SENSE OF ALL OF THESE “REGS”?

The following outline summarizes the federal regulations responsible for environmental compliance:

- A. Title 40 Code of Federal Regulations (40 CFR): This is the ultimate authority for environmental compliance. New editions of 40 CFR are published annually and are available on the World Wide Web. This resource includes chapters on air, water, pesticide, radiation protection, noise abatement, ocean dumping, solid wastes, Superfund, Emergency Planning and Right-to-Know,

effluent guidelines and standards, energy policy, and toxic substances among other topics.

- B. Government regulations administered by the Environmental Protection Agency (EPA)
1. Resource Conservation and Recovery Act (RCRA): Passage of the RCRA in 1976 gave the EPA authority to oversee waste disposal and hazardous waste management. Identification of a waste as being *hazardous* relies on specific analytical tests. Analytical methods that deal with RCRA are found in a collection of four volumes titled “Test Methods for Evaluating Solid Waste Physical/Chemical Methods,” commonly referred to as “SW-846.” Three major subtitles deal with hazardous waste management, solid waste management, and underground storage tanks.
 2. Comprehensive Environmental Response, Compensation and Liability Act (Superfund) (CERCLA): This authority granted to the EPA enables the agency to take short-term or emergency action to address hazardous situations that affect health. The release of the toxic chemical isocyanate in the Bhopal, India community that left over 3000 dead might have fallen under CERCLA if it had occurred in the United States. In addition, the CERCLA contains the authority to force the cleanup of hazardous waste sites that have been identified based on environmental analytical results and placed on the National Priority List. The EPA also has authority to investigate the origins of waste found at these sites and to force the generators and other responsible parties to pay under CERCLA. Analytical methods that deal with CERCLA are provided through the Contract Laboratory Program (CLP). The actual methods are found in various Statements of Work (SOW) that are distributed to qualified laboratories.
 3. Drinking Water and Wastewater: The Safe Drinking Water Act last amended in 1986 gives EPA the authority to regulate drinking water quality. Two types of chemical compounds called *targeted analytes* are considered in this act. The first is the National Primary Drinking Water Standards. These chemical substances affect human health, and all drinking water systems are required to reduce their presence to below the MCL set for each compound by the federal government. The second is the National Secondary Drinking Water Standards. These analytes include chemical substances that

affect the taste, odor, color, and other non health-related qualities of water. A given chemical compound may appear on both lists at different levels of action. The primary drinking water monitoring contaminants are listed in Tables 1.1–1.3 by category. The secondary drinking water monitoring contaminants are listed in Table 1.4. The MCL is listed as well as the method detection limit (MDL). Note that the MDL should always *be less than* the MCL. This was not always true historically in the field of TEQA because available technology always serves to limit the MDL, whereas ecological and toxicological considerations govern the decisions to estimate what makes for an environmentally acceptable MCL.

The Clean Water Act that was last amended in 1987 provides for grants to municipalities to build and upgrade treatment facilities. The act also establishes a permit system known as the National Pollutant Discharge and Elimination System (NPDES) for discharge of natural water bodies by industry and municipalities. Over two-thirds

TABLE 1.1 Primary Drinking Water Monitoring Requirements for Inorganics

Contaminant	MCL (mg/L)	MDL (mg/L)
Antimony	0.006	0.0008–0.003
Arsenic	0.05	
Barium	2	0.001–0.1
Beryllium	0.004	0.00002–0.0003
Cadmium	0.005	0.0001–0.001
Chromium	0.1	0.001–0.007
Copper	1.3	0.001–0.05
Cyanide	0.2	0.005–0.02
Fluoride	4	
Lead	0.015	0.001
Mercury	0.002	0.0002
Nickel	1.1	0.0006–0.005
Nitrate-N	10	0.01–1
Nitrite-N	1	0.004–0.05
Selenium	0.05	0.002
Sodium	20	
Thallium	0.002	0.0007–0.001

TABLE 1.2 Primary Drinking Water Monitoring Requirements for Semivolatile Organics

Contaminant	MCL (mg/L)	MDL (mg/L)
Adipates	0.4	0.006
Alachlor	0.002	0.002
Atrazine	0.003	0.001
Carbofuran	0.04	0.009
Chlordane	0.002	0.0002
Dalapon	0.2	0.001
Dibromochloropropane	0.0002	0.00002
2,4-D	0.07	0.001
Dinoseb	0.007	0.0002
Diquat	0.02	0.0004
Endothall	0.1	0.009
Endrin	0.002	0.00001
Ethylene dibromide	0.00005	0.00001
Glyphosate	0.7	0.006
Heptachlor	0.0004	0.0004
Hexachlorobenzene	0.001	0.001
Hexachlorocyclopentadiene	0.05	0.0001
Lindane	0.0002	0.00002
Methoxychlor	0.04	0.0001
Oxamyl	0.2	0.002
PAHs	0.0002	0.00002
Pentachlorophenol	0.001	0.00004
Phthalates	0.006	0.0006
Picloram	0.5	0.0001
PCBs	0.0005	0.0001
Simazine	0.004	0.00007
Toxaphene	0.003	0.001
2,3,7,8-TCDD (dioxin)	0.00000003	0.000000005
2,4,5-TP (Silvex)	0.05	0.0002
Trihalomethanes (total)	0.1	0.0005

of the states have accepted responsibility for administration of the act. The act and its amendments are based on the fact that no one has the right to pollute the navigable waters of the United States. Permits limit the composition and the concentration of pollutants in the discharge. Wastewater effluents are monitored through the NPDES and this analytical testing has been a boon for commercial analytical laboratories. Every industry and wastewater treatment facility that

TABLE 1.3 Primary Drinking Water Monitoring Requirements for Volatile Organics

Contaminant	MCL (mg/L)	MDL (mg/L)
Benzene	0.005	0.0005
Carbon tetrachloride	0.005	0.0005
Chlorobenzene	0.1	0.0005
<i>p</i> -Dichlorobenzene	0.075	0.0005
<i>o</i> -Dichlorobenzene	0.6	0.0005
1,2-Dichloroethane	0.005	0.0005
1,1-Dichloroethylene	0.007	0.0005
<i>Cis</i> -1,2-dichloroethylene	0.07	0.0005
<i>Trans</i> -1,2-dichloroethylene	0.1	0.0005
Dichloromethane	0.005	0.0005
1,2-Dichloropropane	0.005	0.0005
Ethylbenzene	0.7	0.0005
Styrene	0.1	0.0005
Tetrachloroethylene (PCE)	0.005	0.0005
Toluene	1	0.0005
1,2,4-Trichlorobenzene	0.005	0.0005
1,1,1-Trichloroethane	0.2	0.0005
1,1,2-Trichloroethane	0.2	0.0005
Trichloroethylene (TCE)	0.005	0.0005
Vinyl chloride	0.002	0.0005
Total xylene	10	0.0005

directs into a receiving stream or river has either a federal or state NPDES permit. Methods of analysis are given in 40 CFR 136 for the following:

- (1) Conventional pollutants such as BOD, COD, pH, total suspended solids, oil and grease, and fecal coliforms
- (2) Nonconventional pollutants such as nitrogen, phosphorus, and ammonia
- (3) The 129 so-called priority pollutants.
These pollutants are listed in Tables 1.1, 1.2 and 1.3.
4. Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA): This act requires the EPA to oversee the manufacture and use of chemical substances that directly terminate insects, fungus, and rodents. Analytical methods are required for the analysis of residues in air, soil, and water. Specifications for labeling and warnings are also required under this act.

TABLE 1.4 Secondary Drinking Water Monitoring Requirements

Contaminant	MCL (mg/L)
Aluminum	0.05–0.2
Chloride	250
Color	15 color units
Copper	1.0
Corrosivity	Noncorrosive
Fluoride	2.0
Foaming agents	0.5
Iron	0.3
Manganese	0.05
Odor	3 TON
pH	6.5–8.5
Silver	0.1
Sulfate	250
Total dissolved solids (TDS)	500
Zinc	5

- 5. Food, Drug and Cosmetic Act: Administered by the Food and Drug Administration (FDA), this act governs all chemicals added to foods, drugs, and cosmetics. These substances are not directly under EPA oversight; however, the wastes and by-products generated from the manufacture of these substances are controlled by EPA.
- 6. Toxic Substance Control Act (TSCA): Since 1976, the EPA has been given the authority to gather information on the toxicity and hazardous nature of individual chemicals. Chemical producers are required to supply information dealing with risk assessment of proposed products 90 days before proposed manufacture or import. Included in this information is chemical fate testing, environmental effects testing, and health effects testing.
- 7. Superfund Amendments and Reauthorization Act (SARA): Passed in 1986, this act extends the lifetime of the legislation begun in CERCLA and gives the EPA the authority to remediate a site if no responsible parties can be found to pay.
- 8. Clean Air Act (CAA): The CAA amendments passed in 1990 gave the EPA authority to regulate many hazardous air pollutants (HAPs). Over 100 of these HAPs must be regulated;

TABLE 1.5 Method Series 0000–9999: Test Methods for Evaluating Solid Wastes Physical/Chemical Methods (SW-846, 3rd Edition, Supplement III Update, July, 1997)

Method	Description
0000's	Air emission sampling methods from stationary sources
1000's	Methods to determine hazardous waste characteristics: ignitability, corrosivity, and reactivity; includes the toxicity characteristic leaching procedure (TCLP)
2000's	Unused
3000's	Sample preparation methods for inorganics and organics
4000's	Soil screening methods by immunoassay
5000's	Sample preparation methods for volatile organics and miscellaneous sample preparation methods
6000's	Methods to determine more than one metal at a time
7000's	Methods to determine one metal at a time
8000's	Methods to determine organics
9000's	Miscellaneous test methods and includes cyanides, sulfides, phenols, oil and grease, chlorine, total coliform, potentiometric halides and cyanide, colorimetric chloride and radium-228 isotope

they include well-known organic compounds such as acetaldehyde, benzene, carbon tetrachloride, 1,4-dioxane, hexane, methyl methacrylate, and so forth. The CAA also requires the EPA to establish permits for the regulation of the maximum amounts of emissions by various industries in a manner that is similar to the NPDES program for wastewater effluents.

As if this number of regulations is not enough, many environmental testing labs also have to satisfy state requirements. Most states run their own private lab certification programs. Also, some of the method details differ between state and federal programs. One example from the author's own experience working in an EPA contract lab during the late 1980s involves the implementation of EPA Method 8270 for semivolatile organics in solid waste. EPA Method 3640A, Gel Permeation Chromatography (GPC), is added to any 1 of the 3500 series of sample preparation methods particularly if soil samples are saturated with oil. To satisfy the New Jersey

Department of Environmental Protection requirements when this method is implemented requires that the GPC cleanup step *not be used* even if some soil samples that arrive to the laboratory are indeed saturated with oil. On the other hand, if similar oil-laden soil samples were analyzed for the New York Department of Environmental Conservation, GPC cleanup is acceptable. What is an analyst to do?

5. WHAT ANALYTICAL METHODS SATISFY THESE REGULATIONS?

Today, myriads of analytical methods exist which the above-cited regulations use to demonstrate compliance. The two parameters cited in Tables 1.1–1.3, namely the MCL and MDL, are only obtained with good accuracy and precision by applying all of the skills and techniques of TEQA. The analyst who is about to work in the environmental testing laboratory must have access to these written methods in order to become familiar with their content before he or she can effectively execute the methods themselves. The EPA during the past 30 years has operated within 2 broad mental frameworks or paradigms. The first paradigm that began in the early 1970s and lasted until the early 1990s suggested that the methods written by the agency were the only ones that a laboratory could use in order to satisfy the “regs.” This mindset led to the proverbial “EPA approved” stamp of approval. Instrument manufacturers during the late 1980s would incorporate into their advertisements that their product was EPA approved. During these years, instrument vendors sought collaborations with various EPA offices in an attempt to accelerate the over 10-year delay between proposal of a new method and its acceptance by the EPA.

For example, the development of an instrumental method that would determine more than one inorganic anion in a drinking water sample was announced in 1975 (8). It took until around 1985 for this technique to become incorporated into EPA Method 300.0 as applied to drinking water. Prior to the development of ion chromatography, individual anionic species such as chloride, nitrate, phosphate, and sulfate were determined individually by applying specific colorimetric methods. Each colorimetric method would require separate sample preparation and determinative steps culminating with the use of a spectrophotometer. The idea that a sample could be injected into some kind of chromatograph and all of the ions separated, detected, and quantitated in a reasonable period of time represented a bit of a revolutionary concept! It was also thought that there would be a serious “matrix effect” that would render this new method useless when it came time to analyze “dirty” samples. Today, after some 20 or more years of instrumentation refinement, the advantages and limitations

of the method that requires ion chromatography are well understood (8). This technique has even advanced to the point where one can conduct analyses without having to prepare either an eluent (mobile phase passed through the ion-exchange column) or a regenerate (mobile phase passed through the suppressor), thus further simplifying the implementation of this methodology (9).

Should it take a decade or two for a newly developed method to become “EPA approved”? The future appears not to emulate the past as the second broad mental framework is taking hold; the agency calls it “performance-based method” (PBM).

The new paradigm places more of the responsibility for quality assurance on the individual analyst and on the laboratory rather than on the method itself. It used to be that as long as an analyst followed the recipes (sounds a lot like cooking, doesn't it) in the various methods, then quality data were “assured.” The analyst's input in the process was not required, nor was it requested. This point of view took the analyst out of the “picture” and served to define the analyst more as a robot-technician than as an involved and motivated professional. This point of view left little to no room for analyst intervention. If an analyst wished to skip a step here or there in the method, it was assumed that the method was not followed and, therefore, the data could not be “assured” or, in the parlance of the time, “EPA approved.” PBMs hopefully have and will change this somewhat myopic view of TEQA and, furthermore, begin to place the responsibility on the analyst to yield quality data that are “assured” based on method performance as opposed to blindly adopting the recipe.

For example, instead of an EPA-approved method specifying that a particular gas chromatographic (GC) column be used to measure analyte A in matrix B, the laboratory is free to choose what column to use, as long as an equivalent degree of quality assurance is obtained. In other words, an analytical result for the concentration of analyte A in matrix B from the “approved” method versus the PBM approach should be nearly identical. In addition, the laboratory would not have to conduct the side-by-side comparison of a more conventional sample preparation against a proposed alternative in this new paradigm. For example, it was once considered heresy for an analyst to analyze wastewater samples to determine the slew of organochlorine pesticides using anything except a glass column that contained Supelcoport coated with 1.5% SP-2250 / 1.95% SP-2401 packed into a 1.8-m-long $\times$ 4 mm-inner diameter tube (the essence of Method 608). However, during the late 1980s, labs, including the one this author worked in, began to investigate megabore capillary columns as alternatives to packed columns. Megabore capillary columns made from fused silica could be easily connected to the common $\frac{1}{4}$ -in. injection port used for

packed-column via megabore adapter kits. When combined with element-specific detectors, many new organics could be separated and identified using megabore capillary GC columns (10).

Performance-based methods are an empowering concept. PBMs enable analysts and the laboratories that they work in to become more creative and encourage attempts to develop alternative methods to perform TEQA. Many of these alternative approaches are much more cost-effective while yielding the same outcome. In other words, the performance of the method is the same, a deliverable outcome, and the inputs are much reduced in cost and labor. Alternative methods of sample preparation will be discussed in Chapter 3.

6. HOW ARE THE REGULATORY METHODS FOR TEQA IDENTIFIED?

All EPA methods are classified based on a number, whereas other methods are categorized based on the chemical composition of the analytes of interest. It is useful at this point to briefly outline these EPA method numbers while providing a brief description of their origin (11). All of the major offices within the EPA have had a role in developing analytical methods that bear a number. Series 1–28 are air monitoring methods found in 40 CFR Appendix A, whereas series 101–115 are air monitoring methods in 40 CFR Appendix B. Methods that have three digits refer to methods for the chemical analysis of water and wastes and represent some of the earliest EPA methods. The 100 series of methods characterize the physical properties of water and wastes. The 200 series is devoted to metals and the 300 series deals with inorganics and nonmetals. This is why the ion chromatographic technique discussed earlier is found in this series. The 400 series deals with organics where the emphasis is on nonspecific organics that are primarily found in wastewater. Method 413 for oil and grease, method 415 for total organic carbon, method 420 for total recoverable phenols, and method 425 for methylene blue active surfactants are examples of nonspecific analytical methods that give good indication of whether or not a waste sample contains organics without the need to be specific. These are “work-horse” methods that are usually found in most environmental testing labs.

Method 413 has recently become controversial in the fact that the manufacture of the common extraction solvent used in the method, 1,1,2-trichlorotrifluoroethane (TCTFE), itself a fluorocarbon (Freon 113), is banned. The attributes of TCTFE are such that it enabled this method to move from a purely gravimetric approach, EPA Method 413.1 (Gravimetric, Separatory Funnel Extraction), to an instrumental approach whereby the carbon-to-hydrogen stretching frequency in the infrared spec-

trum enabled a significant reduction in the MDL to occur. The infrared approach was assigned its own method number, EPA Method 413.2 (Spectrophotometric, Infrared). This author's personal opinion is that the more cost-effective infrared approach coupled with the lower MDLs should be maintained. Labs should be strongly encouraged to recycle the waste TCTFE solution so that it can be reused. A simple distillation should suffice to return TCTFE in sufficient purity to use to reextract samples. However, the EPA has chosen to establish Method 1664 for oil and grease analysis. TCTFE has been replaced by hexane and the determination is by weighing the residue after solvent evaporation.

One good thing about this new method is that it is performance based. The analyst can use an alternative approach to oil and grease isolation and recovery. One approach is to use solid-phase extraction (SPE) to isolate the oil and grease, whereas the other approach is to find a way to eliminate use of the solvent. The recently introduced use of infrared cards by 3M Corporation, whereby the oil and grease remains on a thin film of Teflon, is an example of the latter alternative to the conventional liquid-liquid extraction (LLE). The thin film that now offers a fixed path length, is then inserted into the sample holder of a conventional infrared spectrophotometer and the absorbance of the C—H stretching vibration at 2900 cm^{-1} is directly related to concentration via the Beer-Lambert Law of Spectrophotometry discussed in Chapter 4.

The 500 series methods refer to measurement of trace organics in drinking water. This is a collection of methods first published in the late 1980s and incorporated a number of the more innovative techniques introduced at that time. The 600 series methods describe how to isolate, recover, and measure trace organics in wastewaters. These methods were first promulgated in the seventies. Both series of methods measure specific organic compounds and require a means to separate, identify, and quantify these organics. Thus, samples that may contain two or more compounds require a chromatographic separation. Hence, most all methods in these two series are GC methods. The scope of both methods is also limited to organics that are amenable to GC. Sample preparation for trace volatile organics commonly abbreviated VOCs will be introduced in Chapter 3 and the principles of GC will be introduced in Chapter 4.

The 900 series of methods refer to the measurement of radioactivity in drinking water. These methods were first promulgated in 1980.

The four-digit series, methods 0000 to 9999 involve the analysis of solid wastes. This series was promulgated in the early 1980s and has undergone several major revisions up to the present. This series, when compared to all of the others, seems to be the most dynamic and is considered by the EPA as a general guide. These viewpoints infer that these methods were

written within the framework of the second paradigm. In other words, the methods themselves are performance based. The series is periodically updated, and revised and new methods are continuously added. A novice to the field of TEQA would do well to initially focus on this series as a learning tool. A table of contents for this series of analytical methods which is collectively known as SW-846, 3rd edition, is given in Table 1.5 (12). To illustrate the dynamic nature of the SW-846 series of analytical methods for TEQA, consider the very recent development of Method 7473 for determining mercury in various solids of environmental interest (13). A solid sample such as apple leaves, oyster tissue, coal fly ash, or river sediment is dried and thermally decomposed at 750°C in an oxygenated furnace. Mercury vapor is released, swept through, and trapped on a gold amalgamator. The amalgam is thermally desorbed and the Hg vapor is swept into a cold-vapor atomic absorption spectrophotometer where the absorbance is measured at 254 nm. Again, the Beer–Lambert law is used to relate Hg absorbance to concentration. An instrument detection limit (IDL) is reported to be 0.01 ng total Hg.

One of the most challenging methods to implement in the environmental testing laboratory is EPA Method 8270. This is a method that separates, identifies, and quantifies over 100 priority pollutant semivolatile and nonvolatile organics in various solid wastes. The method is a determinative one and is used in combination with one of the sample preparation methods found in the 3000 series of SW-846. A brief discussion of this method follows in order to introduce the reader to the challenges that implementing such a method TEQA bring. The most current SW-846 series lists a third revision of this method identified as 8270C. When Method 8270 was first promulgated, a number of analytes from the targeted list found in the method either did not yield a high recovery or did not chromatograph well. This resulted in the analyst being unable to satisfy the criteria and led to a rejection of the analytical results in the validation process. Method 8270C clearly states at the onset that certain analytes are nondetected by this method or have an unfavorable distribution coefficient (to be discussed in Chapter 3) or adsorb to the inner walls of glassware or hydrolyze during sample preparation. The three revisions of this method serves to illustrate nicely how the EPA has been able to readjust its promulgated methods under the new PBM paradigm to address early flaws. Under the old paradigm, this realization on the part of the EPA was not evident.

The method itself is succinctly summarized as follows:

Method 8270 can be used to quantitate most neutral, acidic or basic organic compounds that are soluble in methylene chloride and capable of being eluted, without derivatization, as sharp peaks from a gas chromatographic fused-silica capillary column coated with a

slightly polar silicone. Such compounds include polynuclear aromatic hydrocarbons, chlorinated hydrocarbons and pesticides, phthalate esters, organophosphate esters, nitrosamines, haloethers, aldehydes, ethers, ketones, anilines, pyridines, quinolines, aromatic nitro compounds and phenols including nitrophenols. (14).

7. WHY IS IT CONSIDERED A CHALLENGE TO IMPLEMENT AN EPA METHOD?

The most tedious and time-consuming aspects of Method 8270 are involved in the preparation and organization of the various referenced chemical standards. This is in addition to the use of reference standards that are required in the various sample preparation methods (i.e., the 3000 series). Method 8270C requires that the following categories of reference standards be prepared, maintained, and replenished when necessary by the analyst:

1. Stock reference standards prepared in the laboratory from the dissolution of neat forms of the individual targeted chemical compounds or purchased from various suppliers. The EPA used to provide these from its repository located in Research Triangle Park, NC, but no longer does.
2. Internal standard reference solutions that contain, with the exception of 1,4-dichlorobenzene- d_8 , five deuterated polycyclic aromatic hydrocarbons, must be prepared. Table 1.6, adapted from the method, shows which of the semivolatile organics are to be quantitated against phenanthrene- d_{10} as the internal standard. The analytes listed in Table 1.6 all elute from the capillary column over a range of retention times that are close to that of phenanthrene- d_{10} .
3. GC-MS Tuning Standard for semivolatiles that contains the tuning compound decafluorotriphenylphosphine (DFTPP) and also 4,4'-DDT, pentachlorophenol, and benzidine. DFTPP is used to verify that the hardware tune was carried out previously and the other three analytes are used to verify injection port inertness and GC column performance. Polar compounds like phenols tend to adsorb to contaminated surfaces and result in the complete loss of response or poor peak shape.
4. Dilution of stock solutions to prepare the series of working calibration standards that are injected into the gas chromatograph-mass spectrometer (GC-MS) to calibrate the instrument for each of the 100 or more semivolatile organics. This series of standards should also contain at least one initial calibration verification

TABLE 1.6 List of Priority Pollutant Semivolatile Organics Quantitated with Respect to the Internal Standard Phenanthrene-d₁₀

4-Aminobiphenyl
Anthracene
4-Bromophenyl phenyl ether
Di- <i>n</i> -butyl phthalate
4,6-Dinitro-2-methylphenol
Diphenylamine
Fluoranthene
Hexachlorobenzene
<i>N</i> -Nitrosodiphenylamine
Pentachlorophenol
Pentachloronitrobenzene
Phenacetin
Phenanthrene
Pronamide

(ICV) standard to assess the accuracy and precision of the calibration curve.

5. Surrogate standard solutions whose analytes are chemically different from any of the targeted analytes and are added to each sample to assess percent recovery.
6. Matrix spike standard solutions and laboratory control standards which are added to one sample per batch to assess matrix effects.
7. System performance check standards and calibration check standards are used to ensure that minimum response factors are met before the calibration curve is used.
8. Blanks which represent standards that contain no targeted analytes, surrogates, or matrix spikes and are used to assess the degree to which a laboratory is contaminated.

All of these standards must be available to the analyst in the laboratory. A most important aspect of an analyst's job is to keep track of all of these standards.

The requirement that the analytes be quantitated using an internal standard mode of instrument calibration is due to the fact that mass spectrometers are intrinsically unstable; that is, their response factor varies with time when compared to other GC detectors such as the flame ionization detector (FID). The internal standard technique of instrument calibration is

discussed in Chapter 2 and the principles of mass spectrometry are introduced in Chapter 4.

8. WHAT OTHER ANALYTICAL METHODS EXIST?

In addition to the methods discussed earlier, a series of ambient air methods, the TO1–TO14 for determining toxic organic compounds is available. Methods IP1A–IP10B is a compendium of methods for determining indoor air pollutants. There is a NIOSH (National Institute for Occupational Safety and Health) *Manual of Analytical Methods* which cover air analysis. Two methods are found in the Code of Federal Regulations: 40 CFR Part 50 for lead, ozone, particulates, and NO_x and 40 CFR 80 for phosphorus and lead in gasoline. The EPA Contract Laboratory Protocol (CLP) Statement of Work for Organics, Dioxins and Inorganics is updated for every contract period. A draft for air analysis exists under the CLP as well. *Standard Methods for the Examination of Water and Wastewater* published by a consortium of the APHA, AWWA, and WEF is a well-regarded so-called “bible” of methods for water and wastewater. The ASTM (American Society for Testing Materials) *Water and Environmental Technology* is updated yearly and is also a valuable resource. *Methods of Air Sampling and Analysis*, 3rd edition from Lewis Publishers and published by an intersociety committee including the American Chemical Society (ACS), Air and Waste Management Association, and other professional societies is also a valuable resource.

The primary and secondary literature of analytical chemistry is a major source of new methods, techniques, variations of older methods, and of innovation. ACS publishes two premier journals, *Analytical Chemistry* and *Environmental Science and Technology*, that should be frequently consulted. The biennial reviews in *Analytical Chemistry* cover topics of interest to TEQA. There exists a host of other very relevant scientific journals and reviews such as the *International Journal of Environmental Analytical Chemistry*, *Analytica Chimica Acta*, *Journal of Chromatography*, *Journal of Chromatographic Science*, *Chemosphere*, and others that contain useful articles. Trade journals that arrive for free to qualified professionals include the following:

- *LC–GC: The Magazine of Separation Science* is must reading for the practicing chromatographer irrespective of whether the reader’s interest is in environmental, pharmaceutical, industrial, academic, or some combination (as is the case for most of us) thereof.
- *American Environmental Laboratory* contains many useful articles that are most relevant to TEQA.

- *Environmental Testing and Analysis* contains many useful articles generally from a private contract lab perspective.
- *American Laboratory* contains many insights that are also relevant to TEQA.
- *Today's Chemist at Work* also contains many useful insights into topics pertinent to TEQA.
- *Spectroscopy* is focused on the practical aspects of atomic and molecular spectroscopy is, at times, also pertinent to TEQA

9. WHAT IS THE PHYSICAL/CHEMICAL BASIS OF THE EPA'S ORGANICS PROTOCOL?

A fundamental question can be asked to the newcomer to all of this: On what basis do all of the 200 or so priority pollutant organics get organized so that a systematic approach to TEQA can be accomplished? In other words, is there a simple and unifying theme that can be introduced to make some sense of all of this? The answer is yes! A flowchart is developed for the protocol as a whole and is depicted in Figure 1.2. Any sample matrix obtained from the environment is first categorized as being either water or soil/sediment and, in turn, appropriate methods are implemented based in large part on the degree of volatility of the respective targeted analytes. Ideally, one would wish to screen samples to obtain a preliminary indication as to whether one must dilute the extract before injection into the determinative instrument. For example, EPA Method 8270C fits very nicely into the scheme shown in Figure 1.3. A sample of soil taken from a Superfund Hazardous Waste Site would be analyzed according to the path for semi-volatile organics. This path assumes that an initial screen is considered, and based on this screen, an appropriate dilution of an extract prepared from the sample made. The sample preparation techniques of LLE and sample cleanup are used and the quantitative determination accomplished using GC-MS. Figure 1.3 is a flowchart for this method and directs the analyst in implementing the method. Note that, along the way, there is ample opportunity for analyst intervention and decision making. Chapters 2–4 introduce those principles responsible for implementing the flowchart schemes shown in Figures 1.2 and 1.3, not only for this particular method but, in general, for all analytical methods.

Before we plunge into the details of analytical methods to measure environmental contaminants, it is important to first consider just how to interpret analytical data. This topic is usually placed either near the end of most books or added in the form of an appendix (15). Increasing emphasis on a more rigorous treatment of analytical data is just beginning to emerge (16).

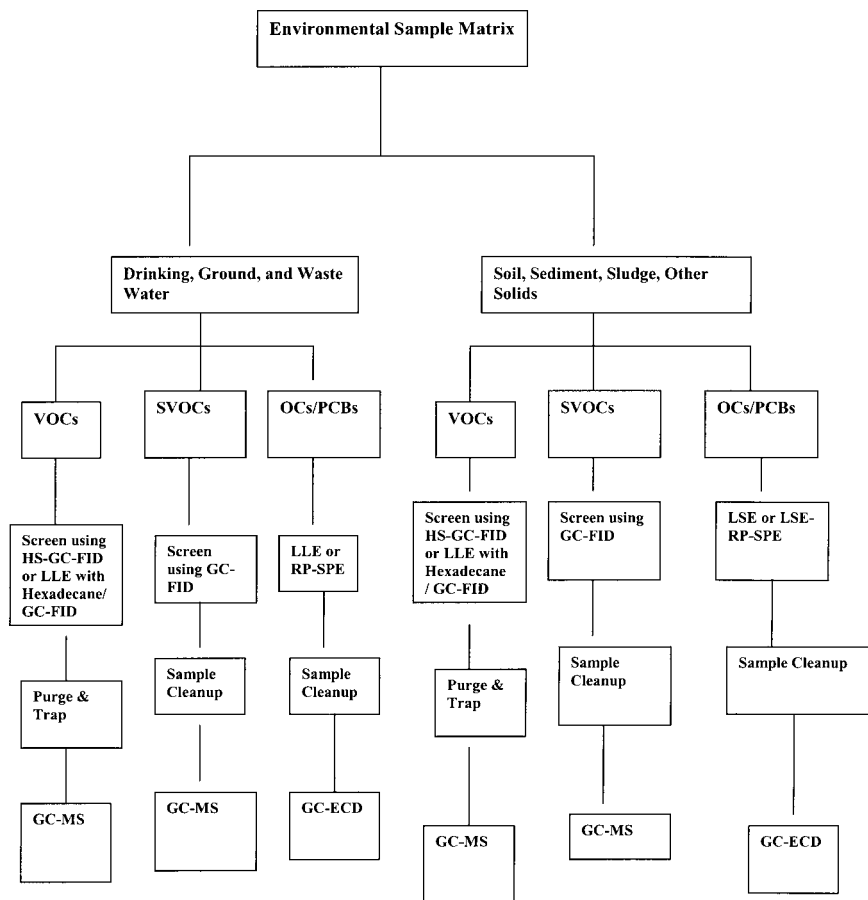


FIGURE 1.2 Overview of the EPA's organics protocol. VOCs, volatile organics; SVOCs, semivolatile organics; OCs/PCBs, organochlorine pesticides/polychlorinated biphenyls; HS-GC-FID, static headspace coupled to gas chromatography and flame ionization detection; LLE, liquid-liquid extraction; LSE, liquid-solvent extraction or solvent leaching from solid matrices; GC-ECD, gas chromatography and electron-capture detection; GC-MS, gas chromatography and mass spectrometry; RP-SPE, reversed-phase solid-phase extraction.

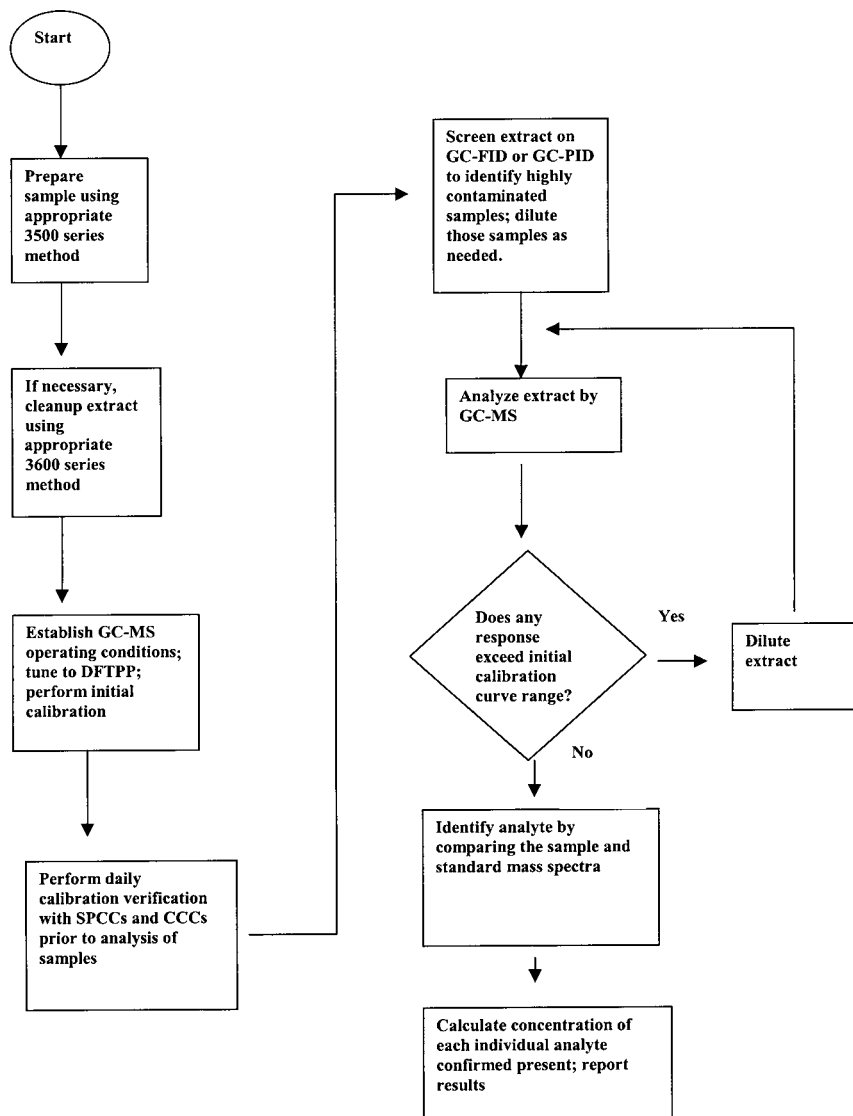


FIGURE 1.3 Flow chart for EPA Method 8270C.

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2

Calibration, Verification, Statistical Treatment of Analytical Data, Detection Limits, and Quality Assurance/Quality Control

Chromatographic and spectroscopic analytical instrumentation are the key determinative tools to quantitate the presence of chemical contaminants in the environment. These instruments generate electrical signals that are related to the amount or concentration of an analyte of environmental significance. This analyte is likely to be found in a sample matrix taken from the environment. Typical sample matrices drawn from the environment include groundwater, surface water, air, soil, wastewater, sediment, sludge, and so forth. Computer technology has merely aided the conversion of an analog signal from the transducer to the digital domain. It is the relationship between the analog or digital output from the instrument and the amount or concentration of a chemical species that is discussed in this chapter. The process by which an electrical signal is transformed to an amount or concentration is called instrument calibration. Chemical analysis based on measuring the mass or volume obtained from chemical reactions is stoichiometric. Gravimetric (where the analyte of interest is weighed) and volumetric (where the analyte of interest is titrated) techniques, are methods that are stoichiometric. Such methods do not require calibration. Most instrumental determinative methods are nonstoichiometric and thus require instrument calibration.

This chapter introduces the most important aspect of TEQA for the reader! After the basics of what constitutes good laboratory practice are discussed, the concept of instrumental calibration is introduced and the mathematics used to establish such calibrations are developed. The uncertainty present in the interpolation of the calibration is then introduced. A comparison is made between the more conventional approach to determining instrument detection limits and the more contemporary approaches that have recently been discussed in the literature (1–6). These more contemporary approaches use least squares regression and incorporate relevant elements from statistics (7). Quality assurance/quality control principles are then introduced. The chapter ends with a comparison of the performance from two hypothetical labs. Every employer wants to hire an analyst who knows of and practices good laboratory behavior.

1. WHAT IS GOOD LABORATORY PRACTICE?

Good Laboratory Practice (GLP) requires that a quality control (QC) protocol for trace environmental analysis be put in place. A good laboratory QC protocol for any laboratory attempting to achieve precise and accurate TEQA requires the following considerations:

- Deciding whether an external standard, internal standard, or standard addition mode of instrument calibration is most appropriate for the intended quantitative analysis application.
- Establishing a calibration curve that relates instrument response to analyte amount or concentration by preparing reference standards and measuring their respective instrument responses.
- Performing a least squares regression analysis on the experimental calibration data to evaluate instrument linearity over a range of concentrations of interest and to establish the best relationship between response and concentration.
- Computing the statistical parameters that assist in specifying the uncertainty of the least squares fit to the experimental data points.
- Running one or more reference standards in at least triplicate as initial calibration verification (ICVs) standards throughout the calibration range. ICVs should be prepared so that their concentrations fall to within the mid-calibration range.
- Computing the statistical parameters for the ICV that assist in specifying the precision and accuracy of the least squares fit to the experimental data points.
- Determining the instrument detection limits (IDLs).

- Determining the method detection limits (MDLs) which requires establishing the percent recovery for a given analyte in both a clean matrix and in the sample matrix. With some techniques, such as static headspace GC, the MDL is identical to the IDL.
- Preparing and running QC reference standards at a frequency of once every 5 or 10 samples. This QC standard serves to monitor instrument precision and accuracy during a batch run. This assumes that both calibration and ICV criteria have been met. A mean value for the QC reference standard should be obtained over all QC standards run in the batch. The standard deviation, s , and the relative standard deviation (RSD) should be calculated.
- Preparing the running QC surrogates, matrix spikes, and, in some cases, matrix spike duplicates per batch of samples. A batch is defined in EPA methods to be approximately 20 samples. These reference standard spikes serve to assess extraction efficiency where applicable. Matrix spikes and duplicates are often required in EPA methods.
- Preparing and running laboratory blanks, laboratory control samples, and field and trip blanks. These blanks serve to assess whether or not samples may have become contaminated during sampling and sample transport.

It has been stated many times by experienced analysts that in order to achieve GLP, close to one QC sample must be prepared and analyzed for nearly each and every real-world environmental sample!

2. HOW IMPORTANT IS INSTRUMENT CALIBRATION AND VERIFICATION?

It is very important and *the* most important task for the analyst who is responsible for operation and maintenance of analytical instrumentation. Calibration is followed by a verification process in which specifications can be established and the analyst can evaluate whether or not the calibration is verified or refuted. A calibration that has been verified can be used in acquiring data from samples for quantitative analysis. A calibration that has been refuted must be repeated until verification is achieved, e.g., if, after establishing a multipoint calibration for benzene via a gas chromatographic determinative method, an analyst then measures the concentration of benzene in a certified reference standard. The analyst expects no greater than a 5% relative error and discovers to his surprise a 200% relative error! In this case, the analyst must reconstruct the calibration and measure the certified reference standard again. Close attention must be paid to those sources of

systematic error in the laboratory that would cause the relative error to greatly exceed the minimally acceptable relative error criteria previously developed for this method.

Three modes of instrument calibration exist: external standard (ES), internal standard (IS), and standard addition (SA). Each mode will be discussed in sufficient detail to enable the reader to acquire a fundamental understanding of the similarities and differences among all three.

2.1 How Does the External Mode of Instrument Calibration Work?

The ES mode uses an external reference source for the analyte whose concentration in an unknown sample is sought. A series of working calibration standards are prepared that encompass the entire range of concentrations anticipated for the unknown samples and may include one or more orders of magnitude. For example, let's assume a concentration of 75 ppb of a trihalomethane (THM) is anticipated in chlorinated drinking water samples. A series of working calibration standards should be prepared whose concentration levels start from a minimum of 5 ppb to a maximum of 500 ppb each THM. The range for this calibration covers two orders of magnitude. Six standards that are prepared at 5, 25, 50, 100, 250, 500 ppb for each THM respectively would be appropriate in this case. Since these standards will not be added to any samples, they are considered "external" to the samples, hence defining this mode as ES. The calibration curve is established by plotting the analyte response against the concentration of analyte for each THM.

The external standard is appropriate when there is little to no matrix effect between standards and samples unless the same matrix is used for preparation of standards. To illustrate this elimination of a matrix effect, consider the situation whereby an aqueous sample is extracted using a non-polar solvent. The reference standard used to construct the ES calibration is usually in an organic solvent such as methanol, hexane, or iso-octane. The analytes of interest are now also in a similar organic solvent. ES is also appropriate when the instrument is stable and the volume of injection of a liquid sample can be reproduced with good precision. A single or multi-point calibration curve is usually established when using this mode.

For a single-point calibration, the concept of a response factor, R_F , becomes important. The use of response factors is valid provided that it can be demonstrated that the calibration curve is, in fact, a straight line. If so, the use of R_F values serves to greatly simplify the process of calibration. R_F is fixed and is independent of the concentration for i th analyte for a truly

linear calibration. A response factor for the i th analyte would be designated as R_{iF} . For example, if 12 analytes are to be calibrated and we are discussing the seventh analyte in this series, i would then equal 7. The magnitude of R_F does indeed depend on the chemical nature of the analyte and on the sensitivity of the particular instrument. The definition of R_F for ES is given as follows:

$$R_F^i = \frac{\Delta A_S^i}{\Delta C_S^i} \quad (2.1)$$

A response factor for each analyte (i.e., the i th analyte), is obtained during the calibration and is found by taking the ratio of the incremental change in peak area for the i th analyte, ΔA_S^i , to the incremental change in concentration of the i th analyte in the reference standard, ΔC_S^i . Quantitative analysis is then carried out by relating the instrument response to the analyte concentration in an unknown sample according to

$$A^i = R_F^i C_{\text{unknown}}^i \quad (2.2)$$

Equation (2.2) is then solved for the concentration of the i th analyte, C_{unknown}^i , in the unknown environmental sample.

Figure 2.1 graphically illustrates the ES approach to multipoint instrument calibration. Six reference standards, each containing Aroclor 1242 (AR 1242), were injected into a gas chromatograph that incorporates a capillary column appropriate to the separation and an electron-capture detector. This instrumental configuration is conveniently abbreviated C-GC-ECD. Aroclor 1242 is a commercially produced mixture of 30 or more polychlorinated biphenyl (PCB) congeners. The peak areas under the chromatographically resolved peaks were integrated and reported as an area count in units of microvolts-seconds ($\mu\text{V}\cdot\text{s}$). The over 30 peak areas are then summed over all of the peaks to yield a total peak area, A_T^{1242} according to

$$A_T^{1242} = \sum_i A^i$$

The total peak area is then plotted against the concentration of Aroclor 1242 expressed in units of parts per million (ppm). The experimental data points closely approximate a straight line. This “closeness of fit” demonstrates that the summation of AR 1242 peak areas varies linearly with AR 1242 concentration expressed in terms of a “total Aroclor”. These data were obtained in the author’s laboratory and nicely illustrates the ES mode. Chromatography processing software is essential to accomplish such a see-

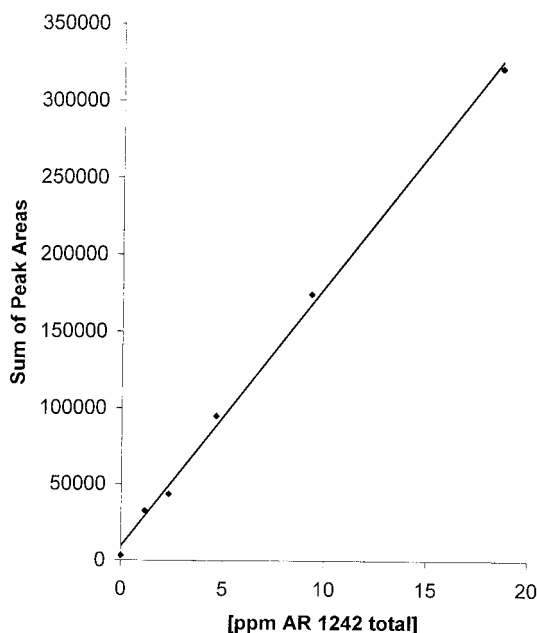


FIGURE 2.1 Calibration for Aroclor 1242 using an external standard.

mingly complex calibration in a reasonable time frame. This author would not want to undertake such a task armed with only a slide rule!

2.2 How Does the IS Mode of Instrument Calibration Work?

The IS mode is most useful when it has been determined that the injection volume cannot be reproduced with good precision. This mode is also preferred when the instrument response for a given analyte at the same concentration will vary over time. Both the analyte response and the IS analyte response will vary to the same extent over time; hence, the ratio of analyte response to IS response will remain constant. The use of an IS thus leads to good precision and accuracy in construction of the calibration curve. The calibration curve is established by plotting the ratio of the analyte to IS response against either the ratio of the concentration of analyte to the concentration of IS or to the concentration of analyte. In our THM example, 1,2-dibromopropane (1,2-DBP) is often used as a suitable IS. The molecular formula for 1,2-DBP is similar to each of the THMs and this results in an instrument response factor that is near to that of the THMs. The concen-

tration of IS in all standards and samples must be identical so that the calibration curve can be correctly interpolated for the quantitative analysis of unknown samples. Refer to the THM example above and consider the concentrations cited above for the six-point working calibration standards. 1,2-DBP is added to each standard so as to be present at, for example, 200 ppb. This mode is defined as such since 1,2-DBP must be present in the sample or is considered as “internal” to the sample. A single-point or multi-point calibration curve is usually established when using this mode.

For a single-point calibration approach, a relative response factor is used and is defined as follows:

$$RR_F^i = \frac{\Delta(A_S^i/A_{IS}^i)}{\Delta(C_S^i/C_{IS}^i)}$$

Quantitative analysis is then carried out by relating the ratio of analyte instrument response for an unknown sample to that of IS instrument response to the ratio of unknown analyte concentration to IS concentration according to

$$\frac{A_{\text{unknown}}^i}{A_{IS}^i} = RR_F^i \frac{C_{\text{unknown}}^i}{C_{IS}^i} \quad (2.3)$$

Equation (2.3) is then solved for the concentration of analyte i in the unknown sample, C_{unknown}^i .

As Eq. (2.3) suggests, the concentration of IS must be the same in all standards and samples. A_{unknown}^i is allowed to vary with time; however, the ratio, $A_{\text{unknown}}^i/A_{IS}^i$ and also RR_F^i remain fixed over time. The IS mode is more likely to be used with instruments whose detectors are of a high-energy nature. One example of this type of instrument is a mass spectrometer. In fact, all EPA methods that require a mass spectrometer use the IS mode of calibration.

Figure 2.2 graphically illustrates the internal standard approach to multipoint instrument calibration for trichloroethylene (TCE) using perchloroethylene (PCE or tetrachloroethylene) as the IS. An automated head-space gas chromatograph incorporating a capillary column and ECD (HS-GC-ECD) was used to generate the data. Figure 2.2 is a plot of $A_S^{\text{TCE}}/A_{IS}^{\text{PCE}}$ versus C_S^{TCE} for a four-point calibration obtained in the author's laboratory. A straight line is then drawn through the experimental data points whose slope is m . Rewriting Eq. (2.3) gives the mathematical equivalent for this calibration plot:

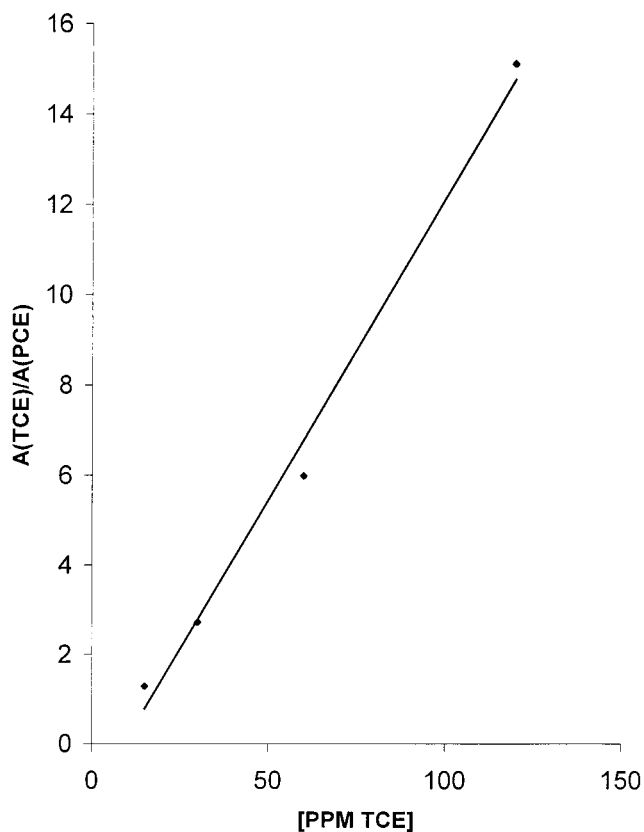


FIGURE 2.2 Calibration for TCE using an internal standard and PCE as the internal standard.

$$\frac{A_S^{\text{TCE}}}{A_{\text{IS}}^{\text{PCE}}} = k C_S^{\text{TCE}}$$

where

$$k = \frac{m}{C_{\text{IS}}^{\text{PCE}}}$$

Quantitative analysis is accomplished by interpolating the calibration curve. This yields a value for the concentration for TCE expressed in units of ppm. The concentration of TCE in a groundwater sample obtained from a

groundwater aquifer that is known to be contaminated with this priority pollutant volatile organic compound (VOC) can then be found.

2.3 HOW DOES THE SA MODE OF INSTRUMENT CALIBRATION WORK?

The SA mode is used primarily when there exists a significant matrix interference and where the concentration of the analyte in the unknown sample is appreciable. SA becomes a calibration mode of choice when the analyte-free matrix cannot be obtained for the preparation of standards for ES. However, for each sample that is to be analyzed, a second, so-called “standard added” or “spiked” sample must also be analyzed. This mode is preferred when trace metals are to be determined in complex sample matrices such as wastewater, sediments, and soils. If the analyte response is linear within the range of concentration levels anticipated for samples, it is not necessary to construct a multipoint calibration. Only two samples need to be measured, the unspiked and spiked sample. Assume that x represents the amount of analyte originally present in an unknown sample and that y represents the amount of standard analyte added. The instrument response is directly related to the concentration of analyte in both the unknown sample and in the spiked sample. We also know that the total concentration C_S is directly proportional to the total response R_S . These relationships are

$$\begin{aligned}C_x &= kR_x \\C_y &= kR_y \\C_S &= k(R_x + R_y) \\R_S &= R_x + R_y\end{aligned}$$

By taking the ratio of C_x to C_y and simplifying, we obtain

$$\frac{C_x}{C_y} = \frac{R_x}{(R_S - R_x)}$$

For real samples that may have nonzero blanks, the concentration of analyte in an unknown sample, C_x , can be found knowing only the measurable parameters R_S and R_x and instrument responses in blanks and the estimated concentration of a single “standard added” or “spike”, C_y , according to

$$C_x = \frac{C_y(R_x - R_{xb})}{(R_S - R_x) - (R_{Sb} - R_{xb})}$$

where R_X is the instrument response for the unknown sample, R_{Xb} is the instrument response for a blank that is associated with the unknown sample, R_S is the instrument response for the spike or standard addition sample and includes both the response from the analyte originally in the sample and the response from the added analyte. R_{Sb} is the instrument response for a blank associated with the standard addition sample and accounts for any contribution that the spike makes to the blank.

3. WHAT DOES “LEAST SQUARES REGRESSION” REALLY MEAN?

Ideally, a calibration curve that is within the linearity range of the instrument's detector exhibits a straight line whose slope is constant throughout the range of concentrations taken. By minimizing the sum of the squares of the residuals, a straight line with a slope m and a y intercept b is obtained. This mathematical approach is called a least squares fit of a “regression line” to the experimental data. The degree of fit expressed as a “goodness of fit” is obtained by the calculation of a correlation coefficient. The degree to which the least squares fit reliably relates detector response and analyte concentration can also be determined using statistics. Upon interpolation of the least squares regression line, the concentration or amount of analyte is obtained. The extent of uncertainty in the interpolated concentration or amount of analyte in the unknown sample is also found. In the next section, equations for the least squares regression will be derived and treated statistically to obtain equations that state what degree of confidence can be achieved in an interpolated value. These concepts are at the heart of what constitutes GLP.

3.1 How Do You Derive the Least Squares Regression Equations?

The concept starts with a definition of a residual for the i th calibration point. The residual Q_i is defined to be the square of the difference between the experimental data point y_i^e and the calculated data point from the best fit line y_i^c . Figure 2.3 illustrates a residual from the author's laboratory where a least squares regression line is fitted from the experimental calibration points for *N,N*-dimethyl-2-aminoethanol using gas chromatography. Expressed mathematically,

$$Q_i = |y_i^e - y_i^c|^2$$

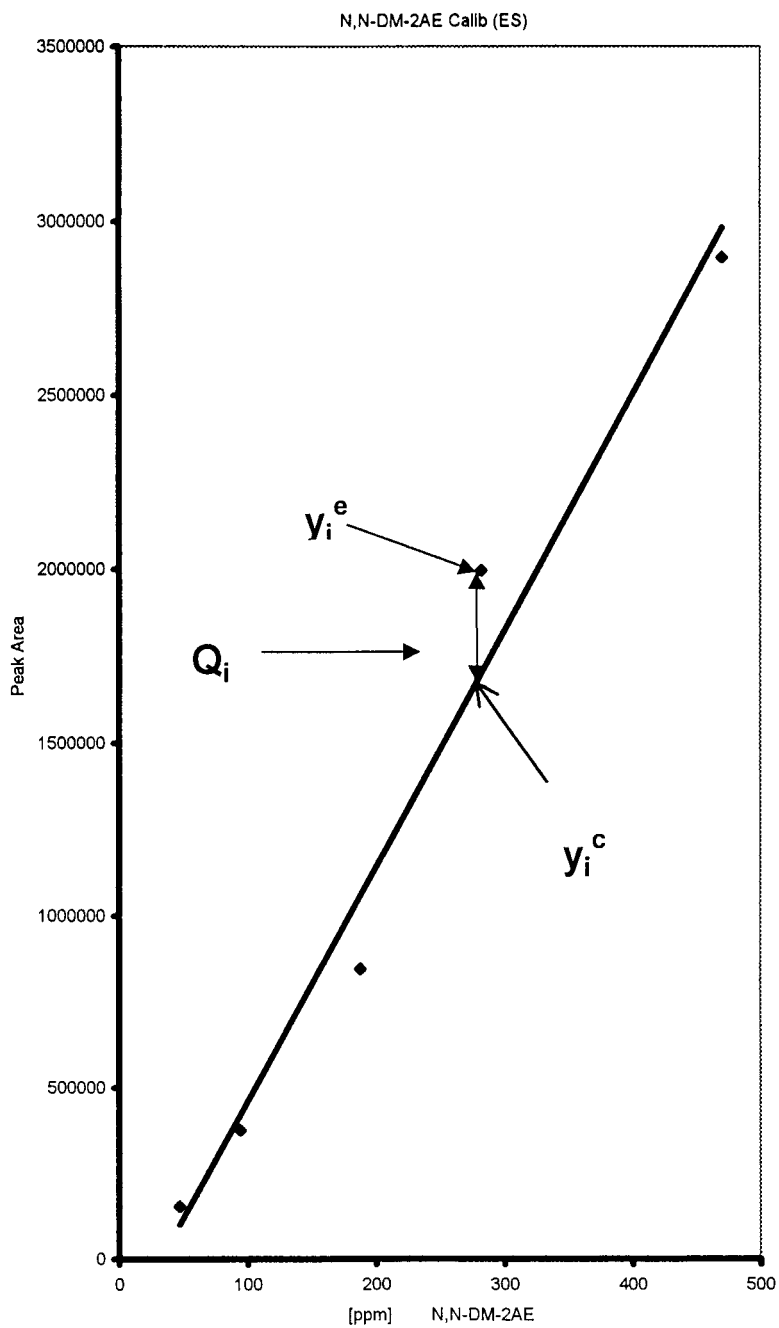


FIGURE 2.3 Experimental versus calculated i th data point.

where y^c is found according to:

$$y_i^c = mx_i + b$$

with m being the slope for the best fit straight line through the data points and b being the y intercept for the best fit straight line. x_i is the amount of analyte i or the concentration of analyte i . x_i is obtained from a knowledge of the analytical reference standard used to prepare the calibration standards and is assumed to be free of error. There are alternative relationships for least squares regression that assume x_i is not free of error. To obtain the least squares regression slope and intercept, the sum of the residuals over all N calibration points, defined as Q , is first considered:

$$Q = \sum_i^N |y_i^e - y_i^c|^2$$

$$Q = \sum_i^N [y_i^e - (mx_i + b)]^2$$

The total residual is now minimized with respect to both the slope m and the intercept b :

$$\frac{\partial Q}{\partial b} = 0 = -2 \sum_i^N [y_i^e - (mx_i + b)] \quad (2.4)$$

$$\frac{\partial Q}{\partial m} = 0 = -2 \sum_i^N x_i [y_i^e - (mx_i + b)] \quad (2.5)$$

Rearranging Eq. (2.4) for b ,

$$b = \frac{1}{N} \left[\sum_i y_i - m \sum_i x_i \right] \quad (2.6)$$

Rearranging Eq. (2.5) for m ,

$$m = \frac{\sum_i x_i y_i - b \sum_i x_i}{\sum_i x_i^2} \quad (2.7)$$

Next, substitute for b from Eq. (2.6) into Eq. (2.7):

$$m = \frac{\sum_i x_i y_i - (1/N)(\sum_i y_i - m \sum_i x_i) \sum_i x_i}{\sum_i x_i^2}$$

Upon simplifying, we obtain

$$m = \frac{N \sum_i x_i y_i - \sum_i x_i \sum_i y_i}{N \sum_i x_i^2 - (\sum_i x_i)^2} \quad (2.8)$$

Recall the definition of a mean:

$$\bar{x} = \frac{\sum_i x_i}{N}, \quad \bar{y} = \frac{\sum_i y_i}{N}$$

rearranging in terms of summations gives:

$$\sum_i x_i = N\bar{x} \quad (2.9)$$

$$\sum_i y_i = N\bar{y} \quad (2.10)$$

Upon substituting Eqs. (2.9) and (2.10) into Eq. (2.8), we arrive at an expression for the least squares slope m in terms of only measurable data points:

$$m = \frac{\sum_i x_i y_i - N\bar{x}\bar{y}}{\sum_i x_i^2 - N\bar{x}^2} \quad (2.11)$$

Defining the sum of the squares of the deviations in x and y calculated from all N pairs of calibration points gives

$$SS_{xx} = \sum_i^N (x_i - \bar{x})^2$$

$$SS_{yy} = \sum_i^N (y_i - \bar{y})^2$$

The sum of the products of the deviation s is given by

$$SS_{xy} = \sum_i^N (x_i - \bar{x})(y_i - \bar{y})$$

The slope for the least squares regression can then be expressed as

$$m = \frac{SS_{xy}}{SS_{xx}} \quad (2.12)$$

and the y intercept can be obtained by knowing only the slope m and the mean value of all of the x data and the mean value of all of the y data according to

$$b = \bar{y} - m\bar{x} \quad (2.13)$$

Equations (2.12) and (2.13) enable the “best-fit” calibration line to be drawn through the experimental x, y points. Once the slope m and the y intercept b for the least squares regression line are obtained, the calibration line can be drawn by any one of several graphical techniques. A commonly used technique is to use Excel to graphically present the calibration curve. These equations can also be incorporated into computer programs that allow rapid computation.

A quantitative measure of the degree to which the dependent variable (i.e., the analytical signal) depends on the independent variable, the concentration of analyte i , is denoted by the correlation coefficient r according to

$$r = \frac{\sum_i [(x_i - \bar{x})(y_i - \bar{y})]}{\sqrt{[\sum_i (x_i - \bar{x})^2][\sum_i (y_i - \bar{y})^2]}}$$

Using the previously defined terms, the correlation coefficient can be described as

$$r = \frac{SS_{xy}}{\sqrt{SS_{xx}SS_{yy}}} \quad (2.14)$$

As r approaches 1, the correlation is said to approach perfection. A good linear calibration plot can achieve values for r that easily reach and exceed 0.9990 and suggests that good laboratory technique as well as optimum instrument performance have been achieved. A good correlation coefficient should not be confused with the notion of calibration linearity. A curve that upon visual inspection appears to be a nonlinear curve can exhibit a correlation coefficient that is calculated to be quite close to 1. The square of r is called the coefficient of determination. Several commercially available chromatography software packages such as Turbochrom (PE-Nelson) are programmed to calculate only the coefficient of determination following the

establishment of the least squares regression calibration curve. Equation (2.12), which expresses the least squares slope m in terms of a ratio of sums of squares, can be compared to Eq. (2.14). If this is done, it becomes obvious that the true nature of r is merely a scaled version of the slope (i.e., the slope estimate multiplied by a factor to keep r always between -1 and $+1$) (7):

$$r = m \sqrt{\frac{S_{xx}}{S_{yy}}}$$

3.2 To What Extent Are We Confident in the Analytical Results?

How reliable are these least squares parameters? To answer this question, we first need to find the standard deviation about the least squares “best-fit” line by summation over N calibration points of the square of the difference between experimental and calculated detector responses according to

$$s_{y/x} = \sqrt{\frac{\sum_i^N (y_i^e - y_i^c)^2}{N - 2}} \quad (2.15)$$

where $s_{y/x}$ represents the standard deviation of the vertical residuals and $N - 2$ represents the number of degrees of freedom. N is the number of x, y data points used to construct the “best-fit” calibration line less a degree of freedom used in determining the slope and a second degree of freedom used to determine the y intercept.

The uncertainty in both the slope and intercept of the least squares regression line can be found using the following equations to calculate the standard deviation in the slope, s_m , and the standard deviation in the y intercept, s_b :

$$s_m = \frac{s_{y/x}}{\sqrt{\sum_i^N |x_i - \bar{x}|^2}}$$

$$s_b = \frac{s_{y/x} \sum_i^N x_i^2}{N \sum_i^N |x_i - \bar{x}|^2}$$

3.3 How Confident Are We of an Interpolated Result?

For a given instrumental response such as for the unknown, y_0 , the corresponding value x_0 from interpolation of the “best-fit” calibration is obtained and the standard deviation in x_0 can be found according to

$$s_{x_0} = \frac{s_{y/x}}{m} \sqrt{\frac{1}{L} + \frac{1}{N} + \frac{(y_0 - \bar{y})^2}{m^2 \sum_i^N |x_i - \bar{x}|^2}}$$

where s_{x_0} represents the standard deviation in the interpolated value x_0 and L represents the number of replicate measurements of y_0 for a calibration having N , x, y data points.

Upon replacing the summation with S_{xx} , the standard deviation in the interpolated value x_0 yields a most useful expression:

$$s_{x_0} = \frac{s_{y/x}}{m} \sqrt{\frac{1}{L} + \frac{1}{N} + \frac{(y_0 - \bar{y})^2}{m^2 S_{xx}}} \quad (2.16)$$

This form enables computer programs, such as the LSQUARES one in Appendix C, to be written that allow for rapid calculation of s_{x_0} . Equation (2.16) shows that the uncertainty s_{x_0} , in the interpolated value, x_0 , is largely determined by minimizing the ratio of $s_{y/x}$ to m . A small value for $s_{y/x}$ infers good to excellent precision in establishment of the least squares regression line. A large value for m infers good to excellent detector sensitivity. The standard deviation in the interpolated value x_0 is also reduced by making L replicate measurements of the sample. The standard deviation can also be reduced by increasing the number N of calibration standards used to construct the calibration curve.

The determination of x_0 for a given detector response, y_0 , is, of course, *the* most important outcome of trace quantitative analysis. x_0 , together with an estimate of its degree of uncertainty, represents *the ultimate goal of trace quantitative analysis*, that is, it answers the question How much of analyte i is present and how reliable is this number in a given environmental sample? For TEQA, it is usually unlikely that the population mean for this interpolated value $\mu(x_0)$ can ever be known and that the standard deviation in this population mean, $\sigma(x_0)$, can ever be known. TEQA requires the following:

- A determinative technique whereby the concentration of vinyl chloride can be measured by an instrument. It can be assumed that for a

given analyte such as vinyl chloride, repeatedly injected into a gas chromatograph, $\mu(x_0)$ and $\sigma(x_0)$ are known, however,

- The concentration of vinyl chloride in the environmental sample may involve some kind of sample preparation to get the analyte into the appropriate chemical matrix for the application of the appropriate determinative technique and we can assume that both $\mu(x_0)$ and $\sigma(x_0)$ are unknown.

These two constraints require that the confidence limits be found when the standard deviation in the population mean is unknown. This is where the Student's t -statistics have a role to play! Who was "Student"? Anderson has introduced a little history (9):

Unfortunately, we do not know the true standard deviation of our set; we have only an estimate s based on L observations. Thus, the distribution curve for $\bar{x}$ is an estimate of the true distribution curve. A research chemist, W. S. Gosset, worked out the relationship between these estimates of the distribution curve and the true one so that we can use s in place of σ . Gosset was employed by a well-known Irish brewery which did not want its competitors to know it was using statistical methods. However, he was permitted to publish his work under the pen name "Student," and the test for differences in averages based on his work is known as Student's t test. Student's t test assumes that the average is based on data from a normal population.

Because we know s_{x_0} (the standard deviation in the interpolated value x_0), we can use the Student's t -statistics to estimate to what extent x_0 estimates the population mean μ_{x_0} . This can be determined according to

$$\mu_{x_0} = x_0 \pm t_{1-\alpha/2, df} s_{x_0}$$

where $t_{1-\alpha/2, df}$ represents the value for a two-tailed Student's t at the significance level of α for $N - 2$ degrees of freedom (df), where N is the number of x, y data points used to construct the calibration curve. The term $[\pm t_{1-\alpha/2, df} s_{x_0}]$ is called the confidence interval and represents the range of x values within which one can be $(1 - \alpha/2)$ 100% confident that the mean value for x_0 will approximate the population mean μ_{x_0} . Appendix C shows a computer program written in GWBASIC by this author. The program not only finds the least squares regression line through a set of N calibration points but also finds decision and detection limits, and for a given instrument response, y_0 , the program finds x_0 and the confidence interval for μ_{x_0} .

Figure 2.4 graphically illustrates the calibration line obtained from least squares regression analysis and shows the corresponding $(1 - \alpha/2)$ 100% confidence limits both above and below the line. Also illustrated is the interpolation of an instrument response y_0 that yields x_0 along with the confidence interval. It is surprising how many analytical results are reported from environmental testing labs without any reference to the uncertainty in the interpolated concentration of analyte in the unknown sample! Most EPA methods as presently written also do not incorporate this statistical aspect of interpolation. The following quote reinforces this notion (10):

Analytical chemists must always emphasize to the public that *the single most characteristic of any result obtained from one or more analytical measurements is an adequate statement of its uncertainty interval*. Lawyers usually attempt to dispense with uncertainty and

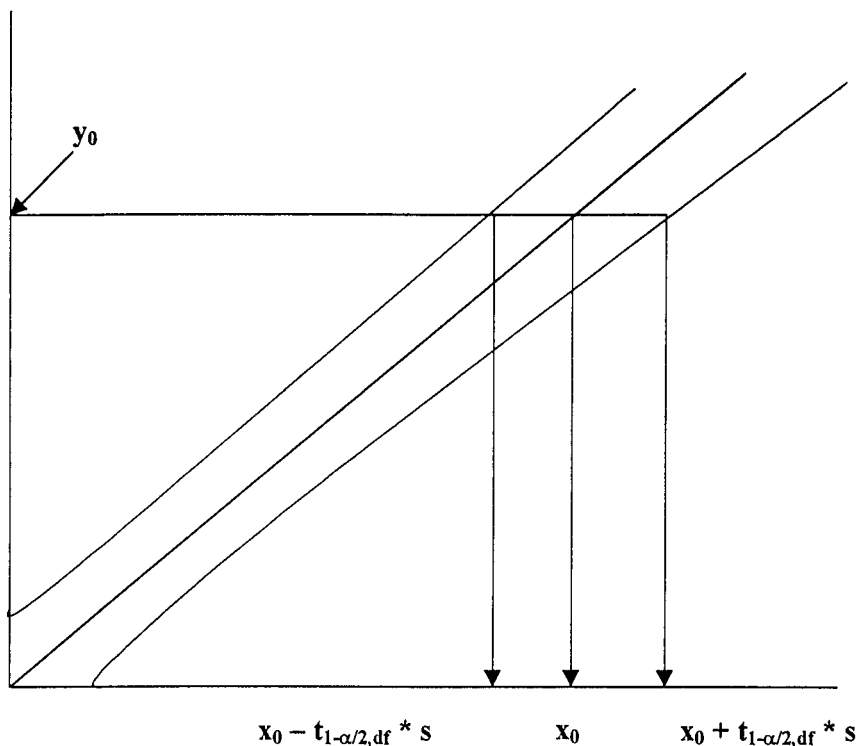


FIGURE 2.4 Interpolation of a least squares regression line showing confidence intervals.

try to obtain unequivocal statements; therefore, an uncertainty interval must be clearly defined in cases involving litigation and/or enforcement proceedings. Otherwise, a value of 1.001 without a specified uncertainty, for example, may be viewed as legally exceeding a permissible level of 1.

The concept of a confidence interval around the least squares calibration line will again become important in the calculation of an instrument's detection limit for a given analyte. In other words, how reliable is this detection limit?

4. HOW DO YOU DERIVE EQUATIONS TO FIND INSTRUMENT DETECTION LIMITS?

The IDL for a specific chemical analyte is defined to be the lowest possible concentration that can be reliably measured. Experimentally, if lower and lower concentrations of a given analyte are measured, the smallest concentration that is "barely" detectable constitutes a rough estimate of the IDL for that analyte using that particular instrument. For years, EPA has required laboratory analysts to first measure a blank a replicate number of times. The most recent guidelines appeared in 40 CFR (Code of Federal Regulations) Part 136, Appendix B (11). The steps that are recommended are listed as follows:

1. Prepare a calibration curve for the test with standards.
2. Analyze seven (7) laboratory water blanks.
3. Record the response of the test for the blanks.
4. Prepare the mean ($\%R_{ave}$) and standard deviation ($s_{\%R}$) of the results from the blanks as above.
5. The IDL is three times the $s_{\%R}$ on the calibration curve.

Fortunately, a bit more thought has been given to the calculation of the IDL than the meager guidelines given above. The average signal that results from these replicate measurements yields a mean signal, S_{blank} . The analyst then calculates the standard deviation of these replicate blanks, s_{blank} , and finds the sum of the mean signal and a multiple k (often $k = 3$) of the standard deviation to obtain a minimum detectable signal S_{IDL} according to (12):

$$S_{IDL} = S_{blank} + ks_{blank}$$

If a least squares calibration curve has been previously established, the equation for this line takes on the common form with S being the instrument

response, m being the least squares slope, and x being the concentration according to

$$S = mx + S_{\text{blank}} \quad (2.17)$$

Solving Eq. (2.17) for x , denoting the IDL as x_{IDL} , and replacing S with this minimum signal, S_{IDL} , yields

$$x_{\text{IDL}} = \frac{S_{\text{IDL}} - S_{\text{blank}}}{m} \quad (2.18)$$

Equation (2.18) represents a “step up” from the meager guidelines introduced earlier; it incorporates the slope of the calibration as a means to address detector sensitivity. It becomes obvious that to minimize x_{IDL} requires that the signal at the detection limit, S_{IDL} , be maximized while the noise level remains minimized and that the steepness of the calibration curve be maximized.

The use of Eq. (2.18) to quantitatively estimate the IDL for chromatographs and for spectrometers has been roundly criticized for more than 10 years. There have been reported numerous attempts to find alternative ways to calculate IDLs. This author will comment on this most controversial topic in the following manner. The approach encompassed in Eq. (2.18) clearly lacks a statistical basis for evaluation and, hence, is mathematically found to be inadequate. As if this indictment is not enough, IDLs calculated based on Eq. (2.18) also ignore the uncertainty inherent in the least squares regression analysis of the experimental calibration as presented earlier. In other words, what if the analyte is reported to be absent when, in fact, it is present (a false negative)? In the subsections that follow, a more contemporary approach to the determination of IDLs is presented and starts first with the concept of confidence intervals about the regression line.

4.1 Can We Derive Equations for Confidence Intervals About the Regression?

The least squares regression line is seen to be shrouded within confidence intervals as shown in Figure 2.4. The derivation of the relationships introduced below was first developed by Hubaux and Vos over 30 years ago (13)! The upper and lower confidence limits for y that define the confidence interval for the calibration are obtained for any x (concentration) and are given as follows:

$$y = [\bar{y} - m(x - \bar{x})] \pm t\sqrt{V_y} \quad (2.19)$$

where $\bar{x}$ and $\bar{y}$ are the respective mean values over all x and y data points and m represents slope of the linear least squares regression. t corresponds to a probability of $1 - \alpha$ for the upper limit and $1 - \beta$ for the lower limit. V_y represents the variance in the instrument response y for a Normal distribution of instrument responses at a given value of x . This equation represents another way to view the least squares regression line and its accompanying confidence interval. The term in brackets is the calculated response based on least squares regression. The variance in y , V_y , is composed of two contributions. The first is the variance in y calculated from the least squares regression and the second is the residual variance σ^2 . Expressed mathematically,

$$V_y = V_{\bar{y}-m[x-\bar{x}]} + \sigma^2 \quad (2.20)$$

and the variance in the least squares calculated y can be viewed as being composed of a variance in the mean value for y and a variance in the x residual according to

$$V_{\bar{y}-m(x-\bar{x})} = V_{\bar{y}} + V_{m|x-\bar{x}|^2}$$

The variance in the least squares regression line can further be broken down into the variance in the mean y expressed as $V_{\bar{y}}$ and the variance in the slope m expressed in terms of V_m according to

$$V_{\bar{y}} = \frac{\sigma^2}{N} \quad (2.21)$$

$$V_m = \frac{\sigma^2}{\sum_i^N |x - \bar{x}|^2} \quad (2.22)$$

Hence, substituting Eqs. (2.21) and (2.22) into Eq. 2.20 gives

$$V_y = \sigma^2 + \frac{\sigma^2}{N} + \frac{\sigma^2 |x - \bar{x}|^2}{\sum_i^N |x_i - \bar{x}|^2} \quad (2.23)$$

Factoring out σ^2 gives

$$V_y = \sigma^2 \left[1 + \frac{1}{N} + \frac{|x - \bar{x}|^2}{\sum_i^N |x_i - \bar{x}|^2} \right] \quad (2.24)$$

The residual variance σ^2 may be replaced by its estimate s^2 , and upon substituting Eq. (2.24) into Eq. (2.19), it gives

$$y = [\bar{y} - m(x - \bar{x})] \pm ts \sqrt{1 + \frac{1}{N} + \frac{|x - \bar{x}|^2}{\sum_i^N |x_i - \bar{x}|^2}} \quad (2.25)$$

Equation (2.25) is an important relationship if one desires to plot the confidence intervals that surround the least squares regression line. The upper confidence limit for the particular case where $x = 0$ is obtained from Eq. (2.25) in which a $1 - \alpha$ probability exists that the Normal distribution of instrument responses falls to within the mean y at $x = 0$. Mathematically, this instrument response y_C , often termed a critical response, is defined as

$$y_C = [\bar{y} - m\bar{x}] + t_{1-\alpha} s \sqrt{1 + \frac{1}{N} + \frac{\bar{x}^2}{\sum_i^N |x_i - \bar{x}|^2}} \quad (2.26)$$

Equation (2.26) can be viewed as being composed of two terms. This is expressed as follows:

$$y_C = y_0 + [P][s]$$

The y intercept of the least squares regression line is given by

$$y_0 = \bar{y} - m\bar{x}$$

The value for y_0 cannot be reduced because it is derived from the least squares regression; however, the second term, $[P][s]$, may be reduced and hence lead to lower IDLs. P is defined as

$$P = t_{1-\alpha} \sqrt{1 + \frac{1}{N} + \frac{\bar{x}^2}{SS_{xx}}}$$

Figure 2.5 provides a graphical view of the terms used to define the decision limit, x_C and detection limit, x_D . The decision limit, x_C , is a specific concentration level for a targeted analyte above which one may decide whether or not the result of analytical measurement indicated detection. The detection limit, x_D , is a specific concentration level for a targeted analyte above which one may rely upon to lead to detection. A third limit, known as a determination limit, or using more recent jargon from the EPA, the practical quantitation limit, is a specific concentration at which

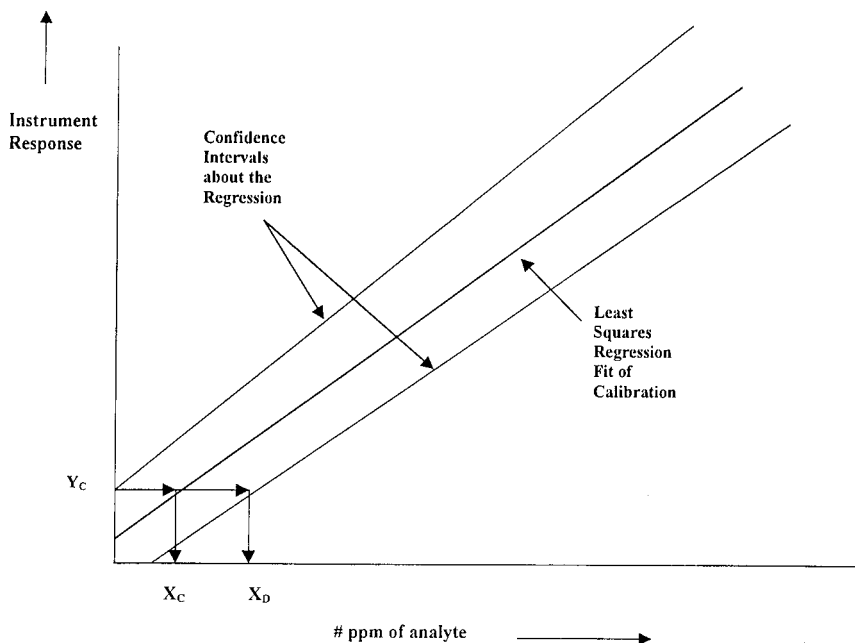


FIGURE 2.5 The linear least squares regression fit to a calibration with statistical evaluation of the goodness of fit.

a given procedure is sufficiently precise to yield satisfactory quantitative analysis (14). The determination limit will be introduced in more detail later.

Referring to Figure 2.5, y_C can merely be drawn horizontally until it meets with the lower confidence limit in which a $1 - \beta$ probability exists that the Normal distribution of instrument responses falls to within the mean at this value of x . The ordinate that corresponds to this value of x , x_D , can be found according to

$$y_D = y_C + t_{1-\beta, s} \sqrt{1 + \frac{1}{N} + \frac{|x_D - \bar{x}|^2}{\sum_i^N |x_i - \bar{x}|^2}} \quad (2.27)$$

Equation (2.27) can be viewed as comprising three terms expressed as follows:

$$y_D = y_0 + [P][s] + [Q][s]$$

where

$$Q = t_{1-\beta} \sqrt{1 + \frac{1}{N} + \frac{|x_D - \bar{x}|^2}{\sum_i^N |x_i - \bar{x}|^2}}$$

The IDL is thus seen to be composed of a fixed value, y_0 , which is derived from the nature of the least squares calibration line and the residual standard deviation s . Factors P and Q can be varied so as to minimize y_D .

Graphically, it is straightforward to interpolate from y_C to the critical limit, x_C , and to interpolate from y_D to the IDL x_D . Numerically, Eqs. (2.26) and (2.27) can be solved for the critical limit x_C and for the IDL, x_D . The equation for the calibration line can be solved for x as follows:

$$x = \frac{y - y_0}{m}$$

When the instrument response y is set equal to y_C , the concentration that corresponds to a critical concentration limit is defined. This critical level x_C , defined almost 20 years ago (13,14), is a decision limit at which one may decide whether or not the result of an analysis indicates detection. The critical level has a specifically defined false positive (type I) error rate, α , and an undefined false negative (type II) error rate, β . x_C is found according to

$$x_C = \frac{y_C - y_0}{m} = \frac{[P][s]}{m} \quad (2.28)$$

In a similar manner, the IDL that satisfies the statistical criteria discussed is obtained according to

$$x_D = \frac{y_D - y_0}{m} = \frac{[P][s] + [Q][s]}{m} = \frac{s(P + Q)}{m} \quad (2.29)$$

The IDL denoted by x_D and given in Eq. (2.29) is the true concentration at which a given analytical procedure may be relied upon to lead to detection (13,14). A false negative rate can be now defined. A Normal distribution of instrument responses at a concentration of zero and at a concentration x_D overlap at x_C and give rise to α and β (shown later in Figure 2.8).

4.2 Is There a Difference in Simplified Versus Contemporary IDLs?

To conclude this discussion on IDLs, it is useful to compare Eqs. (2.19) and (2.29). Both equations relate x_D to a ratio of a standard deviation to the slope of the least squares regression line multiplied by a factor. In the simplified case, Eq. (2.19), the standard deviation refers to detector noise found in the baseline of a blank reference standard, whereas the standard deviation in the statistical case, Eq. (2.29), refers to the uncertainty in the least squares regression itself. This is an important conceptual difference in estimating IDLs. This difference should be understood and incorporated into all future methods.

4.3 How Do MDLs Differ from IDLs?

Obtaining an instrument's IDL represents only one aspect to the overall estimate of a method's detection limit. The only situation whereby an IDL is the same as a MDL is when there is no transfer of analyte from the environmental phase to a second phase. Direct aqueous injection of a groundwater sample into a GC in which there is no extraction step involved is one example of where MDLs equal IDLs. The removal of trace organic volatiles from an aqueous sample either by the purge and trap technique or by static headspace sampling at elevated temperatures results in nearly 100% efficiency and can also be considered as having the MDL equal the IDL. Such is not the case for semivolatile to nonvolatile organics in environmental samples when some type of sample extraction is employed. In these situations, the MDL is often much less than the IDL because sample preparation methods are designed to concentrate the analyte from the environmental matrix to the solvent matrix. The efficiency of this concentration must also be taken into account. One cannot always assume that a given sample preparation is 100% efficient. In fact, a key ingredient in GLP is to conduct an efficiency study by adding an accurately known amount of analyte to the sample matrix and then measure to what extent the method recovers the analyte. The extent of recovery is often called a percent recovery.

4.4 How Do I Obtain MDLs for My Analytical Method?

The MDL can be found only after the IDL, the concentration factor F , and the percent recovery R (expressed as its decimal equivalent) are experimentally determined for the method. F is the ratio of sample volume V_S to extract volume V_e . Expressed mathematically,

$$x_{\text{MDL}} = \frac{x_{\text{IDL}} F}{R}$$

where

$$F = \frac{V_S}{V_e}$$

The percent recovery ($\%R_i$), can be calculated from measuring the instrument response for the analyte in the spiked matrix denoted by A^i with uncertainty characterized by a standard deviation s_R^i and in a reference control that is known to be 100% recovered, A_c^i , with uncertainty s_c^i . This is mathematically defined according to

$$\%R^i(s_R^i) = \frac{A^i(s^i)}{A_c^i(s_c^i)} \times 100 \quad (2.30)$$

The calculation of R^i involves dividing A^i by A_c^i ; this division requires a propagation of error. A relative standard deviation can be calculated by taking into account the propagation of error for random errors using the equation

$$\frac{s_R^i}{R^i} = \sqrt{\left(\frac{s^i}{A^i}\right)^2 + \left(\frac{s_c^i}{A_c^i}\right)^2} \quad (2.31)$$

Equation (2.31) shows that it is relative errors expressed in terms of variances that must be added to enable a determination of the relative error in the percent recovery calculation using Eq. (2.30).

The uncertainty in a percent recovery determination is often expressed in terms of a percent relative standard deviation that is also called the coefficient of variation. The coefficient of variation is calculated according to

$$\text{Coeff Var} = \left(\frac{s_R^i}{R^i}\right) \times 100 \quad (2.32)$$

If sufficient replicate analytical data are available, the variance and standard deviation for a percent recovery study can be found by pooling the data. For example, consider a thorough percent recovery study using solid-phase extraction (SPE) for the isolation and recovery of the pesticide methoxy-chlor from groundwater. If j replicate injections are made for each of g SPE

extractions conducted, an overall standard deviation for this pooled data, s_{pooled} , can be calculated according to

$$s_{\text{pooled}} = \sqrt{\frac{1}{N - g} \sum_g \sum_j (x_j - \bar{x})^2} \quad (2.33)$$

The analyst is now ready to run samples using the selected method because instrument calibration, instrument verification, and the determination of IDLs and MDLs have all been accomplished.

5. WHY SO MANY REPLICATE MEASUREMENTS?

There is no other measurement science that insists on so many replicate measurements than EPA method-driven TEQA! Earlier, the notion of “analyzing seven lab blanks” was cited as a guideline. In fact, the number seven appears quite frequently throughout EPA methods. This author has been known to quip to students that since the Beatles used “number nine” in their music, the EPA likes to use “number seven” in their methods! The answer goes to the heart of the meaning of the Central Limit Theorem of statistics and attempts to apply its meaning to the above-posed question (15; a number of texts discuss the basics of probability and statistics; this author continuously refers to this text originally used by his wife). How many replicate measurements, L , of a given environmental contaminant such as vinyl chloride are needed to be confident 99% of the time that the estimate of the sample variance is less three times the true (i.e., population variance)? Anderson has considered this and sure enough, in his appendix there exists a table that gives the answer, and, “lo and behold”, the answer is seven (16)!

A few words about the Gaussian or Normal distribution are in order at this point along with the commonly used statistical definitions. Figure 2.6 shows a hypothetical Normal distribution with the apex located at the population mean. It is important to distinguish between a confidence *level* or level of significance and a confidence *interval*. The statement “to be 95% confident that a single analytical result such as x_0 , in the absence of any systematic error (i.e., 95 out of every subsequent 100 measurements), will fall to within $\pm 1.96\sigma$ where σ is the standard deviation in the population mean” address both the confidence level and interval. Confidence intervals are labeled to the left of the Gaussian distribution in Figure 2.6 and indicate width or spread, whereas confidence levels, labeled to the right of the Gaussian distribution, refer to heights. The fraction of a

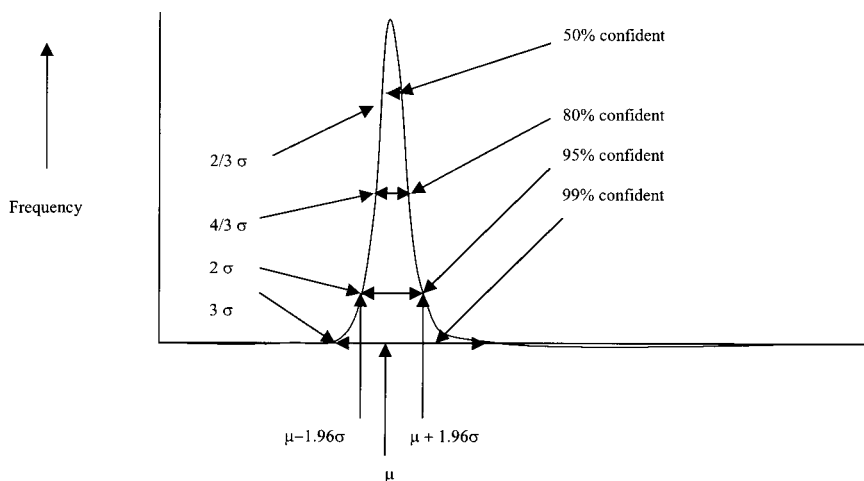


FIGURE 2.6 The Gaussian or Normal distribution with definitions of both the standard deviation and the confidence level.

population of observations dL/L , whose values lie in the region x to $x + dx$ is given by

$$\frac{dL}{L} = \frac{1}{2\pi\sigma} e^{-(x-\mu)^2/2\sigma^2} dx \quad (2.34)$$

Deviations of individual values of x from the mean are conveniently expressed in terms of a dimensionless unit of standard deviation z according to

$$z = \frac{x - \mu}{\sigma}$$

If we differentiate z with respect to x and substitute this into Eq. (2.34), the Gaussian equation looks like

$$\frac{dL}{L} = \frac{1}{2\pi} e^{-z^2/2} dz$$

The population mean can be related to the sample mean for L replicate measurements of a specific chemical contaminant by any of the determinative techniques discussed in Chapter 4 as follows

$$\text{CL for } \mu = \bar{x} \pm \frac{z\sigma}{\sqrt{L}} \quad (2.35)$$

Equation (2.35) suggests that the size of the confidence interval (CL) that surrounds the sample mean can be reduced as we increase L (i.e., make increasing number of replicate measurements). Equation (2.35) is modified when L replicates whose standard deviation s can be found and z is replaced with $t(1 - \alpha/2, \text{df})$ for a two-tailed hypothesis test, where $1 - \alpha/2$ is the confidence level and the number of degrees of freedom where $\text{df} = L - 1$.

6. HOW DO I FIND THE LIMIT OF QUANTITATION?

Again, there are two ways, the simpler and the more contemporary! A recently published text on chemometrics has address this difference as follows (17):

In principle, all performance measures of an analytical procedure... can be derived from a certain critical signal value, y_C . These performance measures are of special interest in trace analysis. The approaches to estimation of these measures may be subdivided into “methods of blank statistics”, which use only blank measurement statistics, and “methods of calibration statistics”, which in addition take into account calibration confidence band statistics.

The simpler approach, in a manner similar to finding the IDL and discussed earlier in this Chapter (and the one suggested by EPA), is to first find the standard deviation in the blank signal; add 10 times this value to the blank signal according to

$$S_{\text{LOQ}} = S_{\text{blank}} + 10s_{\text{blank}} \quad (2.36)$$

and in a manner similar to that shown in Eq. (2.18) that concentration that can be interpolated from S_{LOQ} is defined at the limit of quantitation or practical quantitation limit, x_{LOQ} , according to

$$x_{\text{LOQ}} = \frac{S_{\text{LOQ}} - S_{\text{blank}}}{m} \quad (2.37)$$

The subscript LOQ is the limit of quantitation. This author has attempted to use the standard deviation in the y intercept of the least squares regression of a given calibration for a given analyte of interest [refer to Eq. (2.15) and the equation for s_b] to establish the standard deviation for the blank, S_{blank} ,

in Eq. (2.36). The y intercept itself, b from Eq. (2.13), is used to represent S_{blank} . These two parameters are then substituted into Eq. (2.36) to yield S_{LOQ} . This elevated signal, S_{LOQ} , can be substituted into Eq. (2.36) to find the limit of quantitation.

Oppenheimer et al. (18) have proposed, for the case of the unweighted least squares regression, a relationship similar to that shown in Eq. (2.26) to calculate y_Q according to

$$y_Q = \frac{s_{y/x}}{C} \sqrt{1 + \frac{1}{N} + \frac{\bar{x}^2}{\sum_i^N |x_i - \bar{x}|^2}}$$

where C is the ratio of the standard deviation in the signal corresponding to x_{LOQ} to the signal itself and is usually set equal to 0.10. If y_Q is substituted for S_{LOQ} in Eq. (2.37), x_{LOQ} is found. This is a “calibration statistical” approach to finding the limit of quantitation, x_{LOQ} . Gibbons has defined y_Q as equal to 10 times the standard deviation at the critical level and this term is added to a weighted y intercept to the weighted least squares regression. In addition, a confidence interval is added to yield an alternative minimum level (19).

6.1 Is There a Way to Couple the Blank and Calibration Approaches to Find x_{LOQ} ?

To illustrate the author’s approach, consider the determination of the element Cr at low ppb concentration levels using a graphite furnace atomic absorption determinative technique (determinative techniques are introduced in Chapter 4). The calibration data are as follows:

Concentration (ppb)	Absorbance
1	0.0134
5	0.1282
10	0.2260
15	0.3276
20	0.4567

Entering this calibration data into LSQUARES (refer to Appendix C) yields the following statistical parameters:

Outcome from LSQUARES	Result
Standard deviation in y intercept, $s(b)$	0.0101
Correlation coefficient, r	0.9980
Student's t for one-tail null hypothesis, at a significance level of 95% for 3 degrees of freedom	2.353
Critical response level, y_c	0.0377
Critical concentration level, x_c	1.67 ppb
Detection concentration level, x_D	3.27 ppb

The measured signal from a blank is 0.0620 absorbance units and using the author's program titled LSQUARES to obtain a value for the standard deviation in the least squares regression line, s_b , of 0.0101. Ten times s_b added to 0.0620 gives a $S_{LOQ} = 0.163$. Entering 0.163 into LSQUARES gives 7.22 ± 1.96 ppb Cr as the limit of quantitation. The critical concentration was previously found to be 1.67 ppb Cr, whereas the limit of detection, the IDL for the instrument, was previously found to be 3.27 ppb Cr.

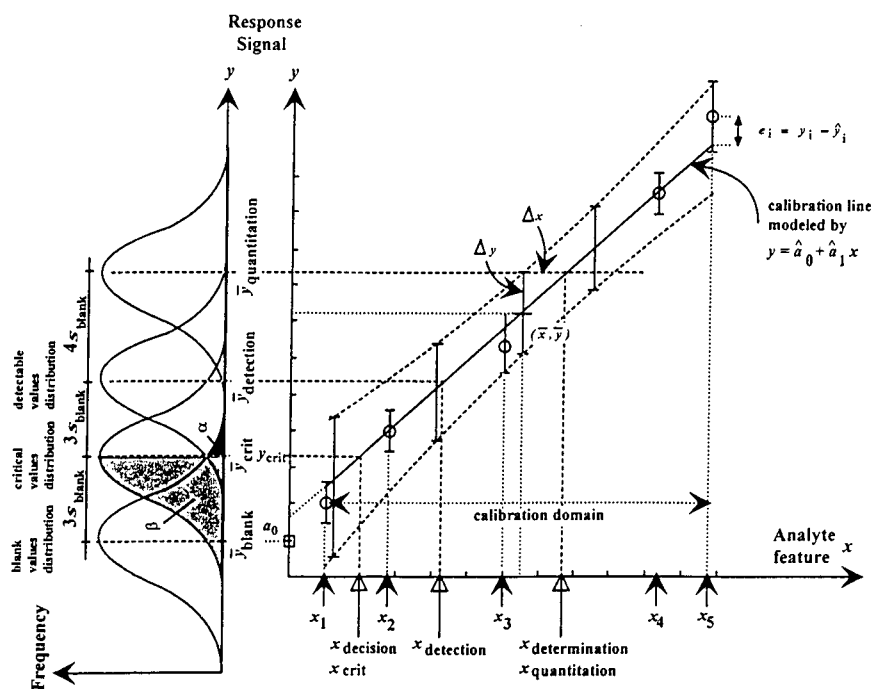


FIGURE 2.7 Blank statistics and confidence band statistics. (From Ref. 20.)

Figure 2.7 is a seemingly complex graph that seeks to show how blank statistics and confidence band statistics can be viewed in an attempt to make sense out of all of this (20)! The confidence interval that surrounds the least squares regression fit through the experimental calibration points is indicated along with extensions to the various Gaussian distributions that appear to the left of the calibration curve. The possibility of committing a significant type II error, represented by β when accepting a detection limit of three times the standard deviation in the blank, S_{blank} , is clearly evident in Figure 2.7. The small type I error at the decision or critical level is evident as well. Note that $y(Q)$ is 10 times the standard deviation in the blank (S_{blank}) and this gives a high degree of certainty that neither type I nor II errors will be committed at the limit of quantitation. This concludes our discussion on the statistical treatment of analytical data for TEQA. We now proceed to a discussion of quality in measurement. Before we do this, let us introduce some definitions and clarify some overused words in the lexicon of TEQA.

7. CAN IMPORTANT TERMS BE CLARIFIED?

Yes they can! Let us start by stating an old adage “samples are analyzed, constituents in samples are identified (qualitative analysis) or determined (quantitative analysis).” According to Erickson, the following definitions can be clarified (21):

- **Technique:** a scientific principle or specific operation, (e.g., gas chromatography–electron-capture detector, GC–ECD, Florisil column cleanup or so-called “Webb and McCall” quantitation.
- **Method:** a distinct adaptation of a technique for a selected measurement purpose (e.g., a specific GC–ECD operating mode for analysis of PCBs, including column specifications and sample preparation.
- **Procedure:** the written directions to use a method or series of methods and techniques; many authors and agencies blur the distinction between “method” and “procedure.”
- **Protocol:** a set of definitive directions that must be followed without deviation to yield acceptable analytical results; analytical protocols may be found as the cookbooks provided within a laboratory derived from common procedures.

The next topic in this chapter introduces the two tenants of Good Laboratory Practice (GLP), quality assurance, and quality control. This is a topic of utmost importance, particularly to laboratory managers and corporate executives! A good lab with a lousy GLP is a sad use of human and facility resources. Each batch of samples that are analyzed must include, in addition to the samples themselves, a matrix spike, a matrix spike dupli-

cate, and one or more laboratory blanks that verify the cleanliness of the laboratory. For the nonstoichiometric chromatographic and spectroscopic determinative techniques (to be discussed in Chapter 4), chemical reference standards must be available and be in high purity as well! These standards are part of GLP and indicate that a laboratory is implementing a quality assurance program.

8. WHAT DOES IT MEAN FOR A LABORATORY TO HAVE A QUALITY ASSURANCE PROGRAM?

An important aspect of GLP is for each laboratory to have a document titled “Quality Assurance” in its possession.

One of the most confusing issues in TEQA involves the need to clearly define the difference between quality assurance (QA), and quality control (QC). QA is a management strategy. It is an integrated system of management activities involving the following five areas:

1. Planning
2. Implementation
3. Assessment
4. Reporting
5. Quality improvement

This plan ensures that a process, item, or service is of the type and quality needed and expected by the client or customer.

Quality control is defined as an overall system of technical activities that measures the attributes and performance of a process, item, or service against defined standards to verify that they meet the stated requirements established by the client or customer. QA is a management function, whereas QC can be viewed as a laboratory function. A laboratory must have a written QA document on file, whereas a record of certified reference standards that are properly maintained implies that QC is in place. Much of what has been discussed in this chapter falls under the definition of QA.

Finally, it is necessary to implement an effective QC protocol when all other aspects of the quantitative analysis have been completed. Two topics emerge from a consideration of EPA-related methods. The first comprises a clear set of definitions of QC reference standards to be used in conducting the quantitative analysis and the second is a set of QC performance criteria and specifications.

9. ARE THERE DEFINITIONS OF QC STANDARDS USED IN THE LAB?

A number of QC standards must be available in the laboratory. These QC standards all have specific roles to play in the QC protocol. The following is a list of the various QC standards including the role of each QC standard as well.

Surrogate Spiking Standard: a solution that contains one or more reference compounds used to assess analyte recovery in the method chosen. Surrogates cannot be targeted analytes. For example, Tetrachloro-*m*-xylene (TCMX) and decachlorobiphenyl (DCBP) dissolved in MeOH are surrogates used in some EPA methods to determine trace organochlorine (OC) pesticide residue analysis. These two analytes are highly chlorinated and are structurally very similar to OCs and PCB targeted analytes. TCMX elutes prior to and DCBP after the OCs and PCBs, thus eliminating any coelution interferences. A fixed volume (aliquot) of this reference solution is added to every sample, matrix spike, blank, blank spike, and control in the protocol.

Matrix Spike Standard: consists of a representative set of the targeted analytes whose percent recoveries are evaluated in the sample matrix. For example, if THMs were the targeted analytes, this reference standard would consist of one or more THMs such as chloroform, bromodichloromethane, and chlorodibromomethane. These compounds would be dissolved in a matrix-compatible solvent such as MeOH. A precise aliquot of this solution is added to a sample so that the effect of the sample matrix on the percent recovery can be evaluated. A second standard called a matrix spike duplicate is often required in EPA methods and is used to assess matrix recovery precision.

Control Standard: consists of the same representative set of targeted analytes used in the matrix spike. The amount of targeted analyte or surrogate is set equal to the amount injected for the surrogate spike and matrix spikes. This amount is dissolved in the exact volume of solvent used in sample preparation. In this way, a standard that assures a 100% recovery is obtained. The ratio of the amount of surrogate analyte to the amount of control analyte is used to determine the analyte percent recovery.

Stock Reference Standard: the highest concentration of target analyte obtained by either dissolving a chemically pure form of the analyte in an appropriate solvent or obtained commercially as a certified reference solution.

Primary Dilution Standard: results from dilution of the stock reference standard.

Secondary Dilution Standard: results from dilution of the primary dilution standard.

Tertiary Dilution Standard: results from dilution of the secondary dilution standard.

Calibration or Working Standards: a series of diluted standards prepared from dilutions made from the tertiary dilution standard. These standards are injected directly into the instrument and used to calibrate the instrument as well as evaluate the range of detector linearity. The range of concentrations should cover the anticipated concentration of targeted analytes in the unknown samples.

Initial Calibration Verification (ICV) Standard: prepared in a similar manner as for the working calibration standards, however, at a different concentration than any of the working standards. Used to evaluate the precision and accuracy for an interpolation of the least squares regression of the calibration curve. This standard is run immediately after the calibration of the working standards.

Laboratory Method Blank: a sample that contains every component used to prepare samples except for the analyte(s) of interest. The method blank is taken through the sample preparation process. It is used to evaluate the extent of laboratory contamination for the targeted analytes.

Trip or Field Blank: a sample that is obtained in the field or prepared during the trip from the field to the laboratory. This blank should contain no chemical contamination, yet should be obtained in a manner similar to the environmentally significant samples themselves.

10. WHAT QC "SPECS" MUST A LAB MEET?

The following five criteria represent five separate QC specifications that should be provided to each and every client who requests analytical services.

10.1 Minimum Demonstration of Capability

Before samples are prepared and analytes quantitatively determined by instrumental analysis, the laboratory must demonstrate that the instrumentation is in sound working order and that targeted analytes can be separated and quantitated. For example, GC operating conditions must be established

and reproducibility in the GC retention times, t_R , for the targeted analytes must be achieved.

10.2 Laboratory Background Contamination

Before samples are prepared and analytes quantitatively determined by instrumental analysis, the laboratory must demonstrate that the sample preparation benchtop area is essentially free of traces of the targeted analyte of interest. This is accomplished by preparing method blanks using either distilled, deionized water, or solvents of ultrahigh purity. This is particularly important for ultratrace analysis [i.e., concentration levels that are down to the low ppb or high ppt (parts per trillion) range]. The use of pesticide residue analysis grade solvents for conducting trace organics analysis and the use of ultratrace nitric and hydrochloric acids for trace metals analysis are strongly recommended. This author believes that there is a considerable number of organics in organic solvents at concentrations in the low ppt level.

10.3 Assessing Targeted and Surrogate Analyte Recovery

Before samples are prepared and quantitatively determined by instrumental analysis, the laboratory must demonstrate that the surrogate and targeted analytes can be recovered to a reasonable degree by the method selected. A rugged analytical method will have an established range of percent recoveries. In TEQA, the value of the percent recovery is said to be “analyte- and matrix-specific.” For example, if EPA Method 625 is used to isolate and recover phenanthracene from wastewater, the percent recovery is acceptable anywhere between 54% and 120%, whereas that for phenol is between 5% and 112%. These percent recoveries are seen to depend on the chemical nature of the analyte (phenanthracene versus phenol) and on the matrix, wastewater. Provost and Elder have put forth a mathematics-based argument stating that the range of percent recoveries that can be expected also depends on the ratio of the amount of added spike to the background concentration present in the environmental sample (22). Table 2.1 applies several mathematical equations discussed in Ref. 22 and clearly shows the impact of spike-to-background ratios on the variability in the percent recovery. Using a spike-to-background ratio of 100 does not change the range of percent recoveries that can be expected. This changes as the ratio is reduced and begins to significantly increase this range when the ratio is reduced to 1 and lower.

TABLE 2.1 Influence of the Spike-to-Background Ratio on Percent Recoveries

Spike/Background	Variance in mean percent recovery	Expected range in percent recoveries ^a		
		$p = 1.0,$ RSD = 0.1	$p = 1.0,$ RSD = 0.2	$p = 0.5,$ RSD = 0.2
Zero background	$(100p)^*(\text{RSD})$	80, 120	60, 140	30, 70
100	$1.02(100p)^*(\text{RSD})$	80, 120	60, 140	30, 70
50	$1.04(100p)^*(\text{RSD})$	80, 120	59, 141	30, 70
10	$1.22(100p)^*(\text{RSD})$	78, 122	56, 144	28, 72
5	$1.48(100p)^*(\text{RSD})$	76, 124	51, 149	26, 74
1	$5.00(100p)^*(\text{RSD})$	55, 145	10, 190	5, 95
0.5	$13.0(100p)^*(\text{RSD})$	28, 170	-44, 240	-22, 122
0.1	$221(100p)^*(\text{RSD})$	-200, 400	-500, 700	-247, 347

^a95% tolerance interval for percent recoveries with assumed values for p and RSD: tolerance limits = $100p \pm \sqrt{\text{Var}(\bar{P})}$.

10.4 Assessing Goodness of Fit of the Experimental Calibration Data and the Range of Linearity

A correlation coefficient, r , of 0.9990 is a goal to achieve between instrument response and analyte amount or concentration for chromatographic and spectroscopic determinative techniques. Over how many orders of magnitude in amount or concentration yields $r = 0.9990$ defines the range of linearity. This range will differ among determinative techniques. Both linear (or first-order least squares regression) and polynomial (second-order and even third-order least squares regression) calibration covering one or more orders of magnitude above the instrument's IDL are all useful ways to address the degree of "goodness of fit." The least squares slope, called a response factor in some EPA methods, and the uncertainty in the slope are also useful criteria in assessing linearity. The coefficient of determination, r^2 , is a measure of the amount of variation in the dependent variable (instrument response) that is accounted for by the independent variable (analyte amount or concentration) (23).

10.5 Assessing ICV Precision and Accuracy

Precision can be evaluated for replicate measurement of the ICV following establishment of the multipoint calibration by calculating the confidence interval for the interpolated value for the ICV concentration. Triplicate

injection of an ICV ($L = 3$) with a concentration well within the linear region of the calibration range enables a mean concentration for the ICV to be calculated. A standard deviation s can be calculated using, for example, Eq. (2.16). One ICV reference standard can be prepared closer to the low end of the calibration range and a second ICV reference standard can be prepared closer to the high end of this range. A confidence interval for the mean of both ICVs can then be found as follows:

$$\text{Conf Int for } \mu_{\text{ICV}} = \bar{x}_{\text{ICV}} \pm \frac{ts}{\sqrt{L}}$$

A relative standard deviation, for the i th component, expressed as a percent (%RSD), also called the *coefficient of variation*, can be calculated based on the ICV instrument responses according to

$$\% \text{RSD}_i^{\text{ICV}} = \frac{s_i^{\text{ICV}}}{\bar{x}_i^{\text{ICV}}} \times 100$$

where s_i is the standard deviation in the interpolated concentration for the i th targeted component based on a multipoint calibration and $\bar{x}$ is the mean concentration from replicate measurements. For example, one can expect a precision for replicate injection into a GC to have a coefficient of variation of about 2%. As another example, one can expect a precision for replicate solid-phase extractions to have a coefficient of variation of between 10% and 20%. The coefficient of variation itself is independent of the magnitude of the amount or concentration of analyte i measured, whereas reporting a mean ICV along with a confidence interval at a level of significance can only be viewed in terms of the amount or concentration measured. The coefficient of variation is thus a more appropriate parameter when comparing the precision between methods.

Accuracy can be evaluated based on a determination of the percent relative error provided that a known value is available. A certified reference standard constitutes such a standard. Accuracy is calculated and reported in terms of a percent relative error according to

$$\% \text{ Error} = \frac{|x_{i(\text{unknown})} - x_{i(\text{known})}|}{x_{i(\text{known})}}$$

Statements of precision and accuracy should be established for the ICV, and as long as subsequent measurements of the ICV remain within the established confidence limits, no new calibration curve needs be generated.

Having discussed what constitutes GLP in terms of QA and QC, let us introduce the very important and not to be neglected “people factor” in all of this.

11. HOW IS AN ENVIRONMENTAL TESTING LAB ORGANIZED?

Laboratories are comprised of people in a workplace using sophisticated technology to generate and report analytical data to clients who have a need for the data. Clearly, people function most effectively under an organization in which each individual knows just what is expected of him or her. Figure 2.8 is a flowchart that depicts the organization of a typical environmental testing laboratory. An individual analyst might work within a lab section that is devoted to the analysis of solid waste samples for the determination of trace levels of semivolatile organics and report to a section manager or supervisor who has had 5 or more years experience in this specialty. A secondary line of responsibility represented by the dashes in Figure 2.8 refers to the QA coordinator’s role in guaranteeing that the lab has adopted a QA plan. For example, our individual analyst may have to report some QC data to the QA coordinator from time to time. The chart in Figure 2.8 facilitates QA and offers the hope that a high level of quality in obtaining analytical data can be maintained. A client can then easily recognize which of two labs, hypothetical Lab X or hypothetical Lab Y, should be offered the contract for environmental monitoring of, perhaps, a hazardous waste site.

12. WHICH LAB WOULD YOU CHOOSE?

Let us suppose that you are a representative working for an environmental consulting firm and you are asked to choose which company, Lab X or Lab Y, should be hired to analyze your contaminated soil samples from this hypothetical hazardous waste site. Let us also assume that the samples come from a Superfund site and that you are interested in the lab providing you with analytical results and interpretation for semivolatile organics using EPA Method 8270C. You receive analytical results from Lab X, and from Lab Y as shown in Table 2.2. Based on your interpretation of the reports from both companies, which lab would you choose to contract with for the analysis? It should be obvious!

Lab X lacks any evidence of QC. Although the lab reported concentration levels of three semivolatile organics in ppb (parts per billion), no statement about the precision of the analysis is provided. No correlation coefficient is provided. The lab’s efficiency in extracting the analytes of

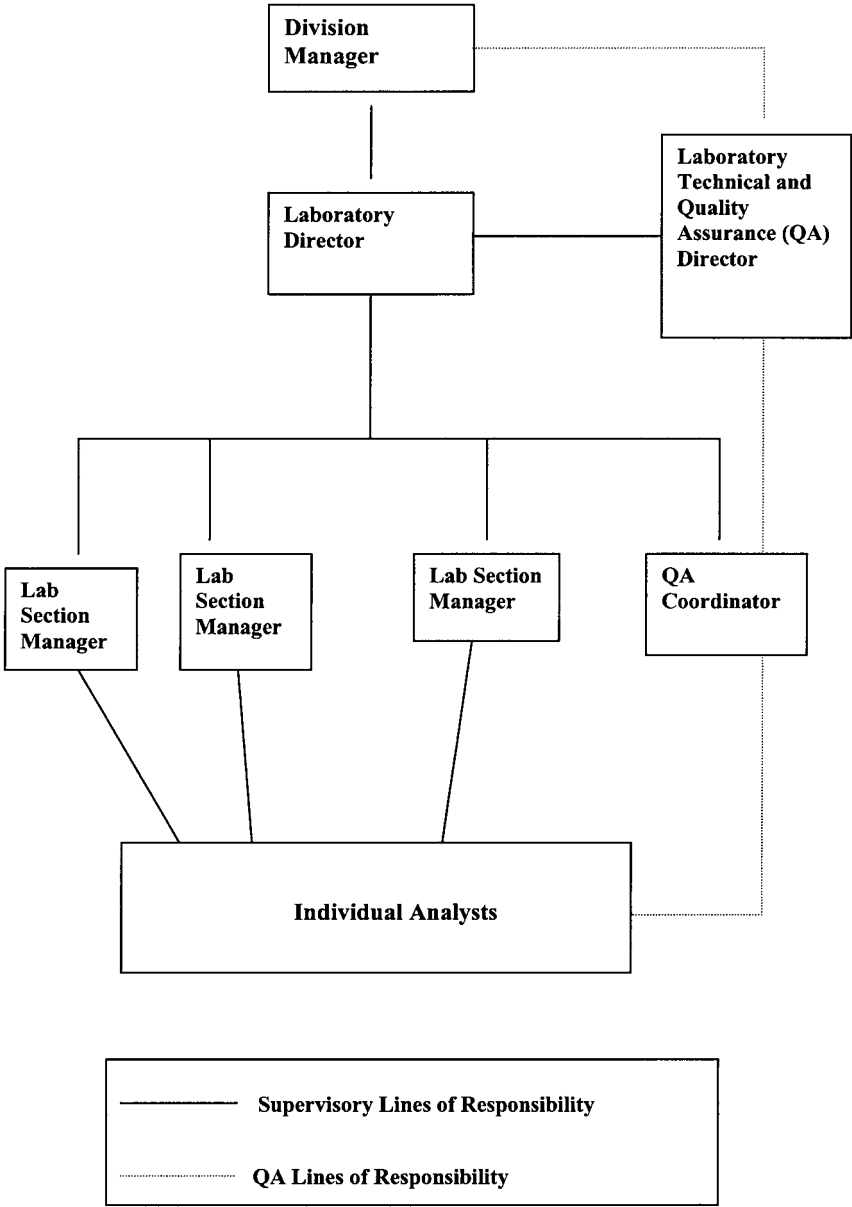


FIGURE 2.8 Typical organization chart of an environmental testing laboratory.

TABLE 2.2 Analytical Results and QC Reports from Labs X and Y

Analyte	ppb	Confidence Interval at 95%	Corr. Coeff.	% Recovery	%RSD in the % Recovery
Company X Analytical Report for the Hazardous Waste Site					
4-Chloro-3-methyl phenol	100	NA	NA	NA	NA
Bis-2-ethylhexyl)phthalate	50	NA	NA	NA	NA
Dibenzo(a,h)anthracene	100	NA	NA	NA	NA
QC Results: none					
Company Y Analytical Report for the Hazardous Waste Site					
4-Chloro-3-methyl phenol	56	10	0.9954	67	15
Bis-2-ethylhexyl)phthalate	47	9	0.9928	84	13
Dibenzo(a,h)anthracene	73	12	0.9959	73	17
QC Results:					
Instrument detection limit (IDL) = 10 ppm					
Method detection limit (MDL) = 10 ppb					
Laboratory blank < MDL					
Field blank < MDL					

Note: NA means not available.

interest from the environmental sample matrix as shown by a percent recovery is not evident in the report. No other QC is reported for this laboratory. This author would immediately ask upon receiving such a report if the QA document is available for review.

Lab Y, on the other hand, not only reported detectable levels of the three organics but also gave a confidence interval for each reported concentration. Lab Y demonstrates that reference standards were run to establish a least squares regression line and the “goodness of fit” expressed by the value of the correlation coefficient. The lab also conducted the required percent recovery studies and demonstrated acceptable precision in the reported % recoveries for each of the three analytes. The lab also reports that it conducted a study of the IDLs and also of the MDLs for the requested method.

13. WHAT SHOULD I KNOW ABOUT SAMPLING?

Historically, the science underlying the phrase “take a representative sample” has not been the province of the analyst. This being said, if a sample is brought to the analyst that is not representative of the contaminated waste site, then anything that the analyst does (the subject of Chapters 3 and 4), is of little value toward achieving the goals of TEQA. We will introduce some basics of sampling but not delve into this in too much depth.

The concentration of any given chemical substance in the environment varies with time. The analyte of environmental interest is found unevenly distributed in the environment. Inhomogeneity (i.e., a lack of a uniformly distributed priority pollutant in the environment) is what leads many to collect numerous samples in the hope that the quantitatively determined analytes can be averaged. Alternatively, a large sample such as a contaminated soil that weighs over 10 kg, defined as a lot or batch, can be subdivided into increments. These increments can be mixed together to give a primary or gross sample. The mass of this gross sample can be further subdivided to yield the secondary sample. The secondary sample then becomes the analytical sample.

The “planning of representative sampling” consists of the following (24):

- Samples must be replicated within each combination of time, location, or other variables of interest.
- An equal number of randomly allocated replicate samples should be taken.
- Samples should be collected in the presence and absence of conditions of interest in order to test the possible effect of the conditions.

- Preliminary sampling provides the basis for the evaluation of sampling design and options for statistical analysis.
- Efficiency and adequacy of the sampling device or method over the range of conditions must be verified.
- Proportional focusing of homogeneous subareas or subspaces is necessary if the entire sampling area has high variability as a result of the environment within the features of interest.
- Sample unit size (representative of the size of the population), densities, and spatial distribution of the objects being sampled must be verified.
- Data must be tested in order to establish the nature of error variation to enable decision on whether the transformation of the data, the utilization of distribution-free statistical analysis procedures, or the test against simulated zero-hypothesis data is necessary.

An “optimized sampling program” must take the following aspects into account (24):

- Place, location, and position of sampling
- Size, quantity, and volume of the sample
- Number of samples
- Date, duration, and frequency of sampling
- Homogeneity of the sample
- Contamination of the sample
- Decontamination of the sample
- Sample conservation and storage

14. ARE THERE MINIMUM “SPECS” FOR SAMPLING?

Yes there are! In the early 1980s, the Committee on Environmental Improvement of the American Chemical Society back suggested a set of minimum sampling requirements to include the following (24):

- Proper statistical design that takes into account the goals of the study and its certainties and uncertainties
- Instructions regarding sample collection, preservation of labeling, and transport to the analytical laboratory
- Training of personnel in the sampling techniques and the procedures specified

According to a recent book on chemometrics in TEQA;

The reader should not, however, forget that the sampling process is very complex and, therefore, that environmental aspects, the ana-

lytical chemistry to be used, and mathematical statistics must be considered simultaneously. (24)

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3

Sample Preparation Techniques to Isolate and Recover Organics and Inorganics

The importance of sample preparation to TEQA is clearly indicated in the following story. This author was once approached by a student during the era when it became apparent that in the 1970s polychlorinated biphenyls (PCBs) had contaminated the striped bass that migrate up the Hudson River in New York to spawn every spring. Once the student learned that a gas chromatograph (GC) is used to measure the extent that fish are contaminated with PCBs and noticed the instrument on the bench in the corner of the laboratory, the student was curious as to exactly how a fish the size of a striped bass could be put into the injection port of the GC. The diameter of the injection port of the GC was less than 1 mm, which, of course, is miniscule in comparison to the size of the fish. The student thought that all that was necessary was to find a way to get the fish into the injection port and the data, which at that time were displayed on a strip-chart recorder, would indicate the extent of this PCB contamination. The student speculated that it might be easier to cut the fish up and attempt to stuff it into the injection port on the GC. Ah, we see for the first time, in this student, a glimpse into the need for sample preparation, bravo!

Indeed, the fish must be transformed in some manner prior to measurement by a determinative technique—in this case, by gas chromatography. Determinative techniques utilize instrumental analysis approaches and

are discussed in Chapter 4. The removal of the PCB from fish tissue (known as the sample matrix) to a form that is compatible with the determinative technique or particular analytical instrument—in this case, the GC—is the basis for sample preparation. The GC requires the introduction of a solvent that contains the dissolved solute—in this case, PCBs. A gas can also be injected into the GC. However, it is much more convenient to get the PCBs from the sample matrix to the liquid state. The liquid is quickly vaporized under the elevated temperature of the GC injection port and undergoes GC separation. The number of molecules of each chemically different substance now present in the vapor causes a perturbation in the GC detector. This perturbation results in an electrical signal whose magnitude becomes proportional to the number of molecules present in the liquid.

This chapter introduces the various techniques that are commonly used to prepare environmental samples and comprises an important component of TEQA. One approach that can be used to “get the striped bass into the machine” to achieve the utmost goal of TEQA (i.e., to isolate, identify, and quantitate the PCB in the sample matrix), defines sample preparation. This chapter starts out with the most common and most conceptually simplistic form of sample preparation whereby a liquid such as water or a solid such as a soil is placed in a beaker or equivalent container. To this container is added an organic solvent that is immiscible with water. The mixture is shaken and allowed to remain stationary for a period of time, such as 15 min. The analytes originally dissolved in the water or adsorbed onto soil particulates are partitioned into the organic solvent. The organic solvent that now contains the dissolved analyte as a solute is referred to as the extractant. After the principles of liquid–liquid extraction (LLE) are introduced and developed, the practice of LLE in its various forms will be discussed.

In addition to LLE, the two other major types of analyte isolation and recovery are solid-phase extraction (SPE) and supercritical fluid extraction (SFE) extraction. SPE refers to those techniques that isolate the analyte from a sample matrix and partition the analytes of interest onto a chemically bonded silica surface. SFE refers to those techniques that isolate the analyte from a sample matrix and partition it into a liquid that has been heated and pressurized beyond its critical temperature and pressure. It is indeed overly simplistic to think that a striped bass can be stuffed into a GC as a means to conduct TEQA!

1. WHAT ARE THE PRINCIPLES UNDERLYING LLE?

A good grounding in the basic principles of LLE is a useful way to begin a chapter that focuses on sample preparation for TEQA. LLE was historically

the first sample preparation technique used in analytical chemistry. Organic chemists have used LLE techniques for over 150 years for isolating organic substances from aqueous solutions. A good definition of LLE has been given earlier in the literature and is stated here:

A substance distributes between two contacting immiscible liquids—water and a suitable organic solvent, for example—roughly in the ratio of its solubility in each if it does not react with either and if it exists in the same form in both. If, at equilibrium, its concentration is much greater in the organic solvent phase than in the aqueous phase, the distribution behavior may be put to analytical use in concentrating the substance into a small volume of the organic liquid and, more importantly, in separating it from substances that do not distribute similarly. (1)

This definition of LLE is concise yet profound in that it covers all ramifications. The first sentence establishes two conditions. Compounds that react with the extractant do not obey the rules. The chemical nature of the compound needs to remain the same throughout the extraction. Mathematical relationships have also been developed to account for the fact that the chemical form may change. This has been called secondary equilibrium effects and this topic will also be introduced in this chapter. The second sentence implies that a concentration factor can be realized. The concentrating nature of LLE is most important to TEQA. The fact that different chemical substances will distribute differently between immiscible liquids also forms the theoretical basis for separation among two or more organic substances that might be initially dissolved in the aqueous solution. These differences are exploited in the design of sample preparation schemes as well as providing for the fundamental basis to explain analyte separation by chromatography. Aqueous solutions are of prime importance to TEQA because our sample matrix, if a liquid, consists of either drinking, surface (i.e., rivers), groundwater, or wastewater obtained from the environment. The fact that the chemical form can change during the extraction process can be exploited in analytical chemistry toward the development of new methods to separate and isolate the analyte of interest.

To understand the most fundamental concept of liquid-liquid extraction, consider placing 100 mL of an aqueous solution that contains 0.1M NaCl and 0.1M acetic acid, HOAc, into a piece of laboratory glassware known as a separatory or commonly abbreviated as a sep funnel (2). This is depicted in Figure 3.1A. Next, 100 mL of diethyl ether, a moderately polar organic solvent that is largely immiscible with water, is added to the funnel. Indeed, some ether will dissolve in water to the extent of 6.89% at 20°C while some water dissolves in the ether to the extent of 1.26% at 20°C (3).

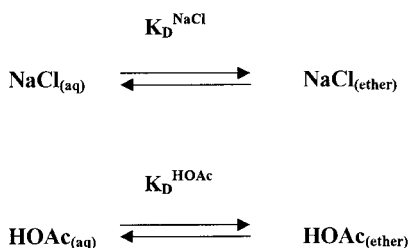
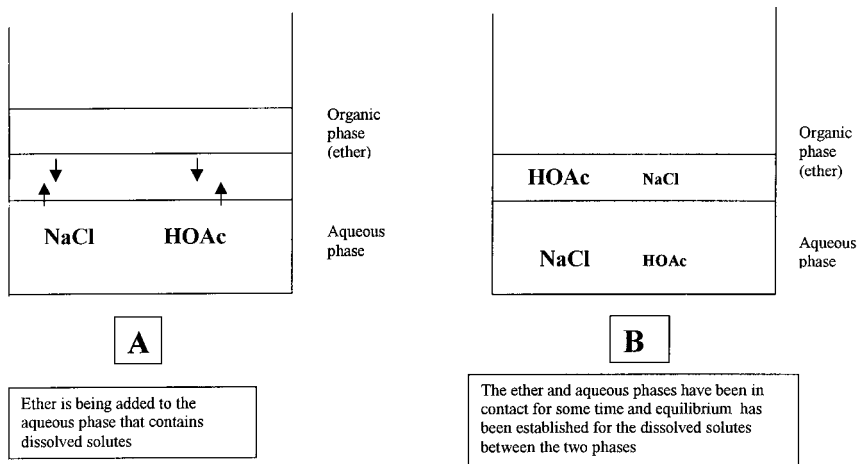


FIGURE 3.1 Hypothetical distribution of solutes NaCl and HOAc between ether and water-immiscible phases.

Upon shaking the contents of the funnel and allowing some time for the two phases to become stationary, the solute composition of each phase is depicted in Figure 3.1B. The lower layer is removed from the sep funnel, thus physically separating the two phases. Upon taking an aliquot (portion thereof) of the ether phase and separately taking an aliquot of the water phase while subjecting this aliquot to chemical analysis reveals a concentration of NaCl, denoted as $[\text{NaCl}]$, at $1.0 \times 10^{-11} M$ and that in water $[\text{NaCl}]_{\text{aq}} = 0.10 M$. Analysis of each phase for acetic acid reveals $[\text{HOAc}]_{\text{ether}} = [\text{HOAc}]_{\text{aq}} = 5 \times 10^{-2} M$. Upon combining both phases again, a second chemical analysis of the composition of each phases reveals exactly the same concentration of HOAc and of NaCl in each phase. As long as the temperature of the two phases in contact with each other of the sep funnel remain fixed, the concentration of each chemical species in both

phases will not change with time. A dynamic chemical equilibrium has been reached. The significant difference in the extent of partitioning of NaCl and of HOAc between diethyl ether and water-immiscible phases can be explained by introducing a thermodynamic viewpoint.

2. DOES THERMODYNAMICS EXPLAIN DIFFERENCES IN NaCl VERSUS HOAc PARTITIONING?

For spontaneous change to occur, the entropy of the universe must increase. The entropy of the universe continues to increase with each and every spontaneous process. LLE represents an ideally closed thermodynamic system in which solutes originally dissolved in an aqueous sample taken from the environment can diffuse across a solvent–water interface and spontaneously partition into the solvent phase. These concepts are succinctly defined in terms of the change in Gibbs free energy, G , for system processes that experience a change in their enthalpy H and in the change in the entropy of the system S . The criteria for spontaneity requires that the Gibbs free energy, G , decrease. In turn, this free-energy change is mathematically related to a system's enthalpy H and entropy S . All three depend on the state of the system and not on the particular pathway, so a change in free energy at constant temperature can be expressed as a difference in the exothermic or endothermic nature of the change and the tendency of the matter in the system to spread according to

$$\Delta G = \Delta H - T \Delta S$$

This equation suggests that for spontaneous physical and/or chemical change to occur, the process proceeds with a decrease in free energy. As applied to phase distribution, equilibrium is reached when the infinitesimal increase in G per infinitesimal increase in the number of moles of solute i added to each phase becomes equal. Hence, the chemical potential of solute i is defined as

$$\mu = \left(\frac{\partial G}{\partial n_i} \right)_{T,P}$$

The chemical potential can also be expressed in terms of a chemical potential under standard state conditions μ^0 and the activity a for a solute in a given phase. Recognizing that a phase that has an activity equal to unity (i.e., $a = 1$ defines the standard state at a given temperature and pressure), the equation for the chemical potential μ for an activity other than $a = 1$ is found according to

$$\mu = \mu^0 + RT \ln a \quad (3.1)$$

Once equilibrium is reached, the net change in μ for the transfer of solute i between phases must be zero so that for our example of NaCl or HOAc in the ether/water-immiscible phase illustration, the chemical potentials are equal:

$$\mu_{\text{ether}}^{\text{NaCl}} = \mu_{\text{aq}}^{\text{NaCl}} \quad (3.2)$$

Hence, upon substituting Eq. (3.1) into Eq. (3.2) for solute i ,

$$\mu_{\text{ether}}^0 + RT \ln a_{\text{ether}} = \mu_{\text{aq}}^0 + RT \ln a_{\text{aq}}$$

which rearranges to

$$RT \ln \left(\frac{a_{\text{ether}}}{a_{\text{aq}}} \right) = \mu_{\text{aq}}^0 - \mu_{\text{ether}}^0 \quad (3.3)$$

The change in standard state chemical potential, $\Delta\mu^0$, is usually expressed as the difference between the organic phase and the aqueous phase according to

$$\Delta\mu^0 = \mu_{\text{ether}}^0 - \mu_{\text{aq}}^0$$

Solving Eq. (3.3) for the ratio of solute activities gives

$$\frac{a_{\text{ether}}}{a_{\text{aq}}} = e^{-\Delta\mu^0}$$

Because $\Delta\mu^0$ is the difference of two constant standard state chemical potentials, it must be a constant. The ratio of activities of NaCl or HOAc is fixed provided that the temperature and pressure are held constant.

A thermodynamic approach has just been used to show what is important analytically, that is, LLE enables an analyte to be transferred from the sample to the extracting solvent and remain at a fixed concentration over time in the extractant. This ratio of activities is defined as the thermodynamic distribution constant, K^0 , so that

$$K^0 \equiv \frac{a_{\text{ether}}}{a_{\text{aq}}} \quad (3.4)$$

3. WHAT ARE SOLUTE ACTIVITIES ANYWAY?

A solute dissolved in a solvent such as water is only partly characterized by its concentration. Solute concentration can be expressed in one of any number of units. The most commonly used units include the following: moles solute per liter solution or molarity (M); moles solute/1000 g water or molality, m ; millimoles solute per liter solution or millimolarity (mM). Those units that have greater relevance to TEQA include the following: mg solute per liter solution or parts per million (ppm); micrograms solute per liter solution or parts per billion (ppb); picograms solute per liter solution or parts per trillion (ppt). Note that TEQA relies exclusively on expressing solute concentration in terms of a weight per unit volume basis. The fact that equilibrium constants in chemistry depend not only on solute concentration but also on solute activities serves to explain why any discussion of distribution equilibria must incorporate solute activities. Solute activities are introduced in any number of texts (4).^{*} Activities become important when the concentration of an electrolyte in an aqueous solution becomes appreciable (i.e., at solute concentrations of $0.01M$ and higher).

The extent to which a solution whose concentration of solute i contributes to some physical/chemical property of this solution (i.e., its activity, a_i) is governed by the solute's activity coefficient γ_i according to

$$a_i = \gamma_i c_i$$

Activity coefficients strongly depend on the ionic strength of a solution. Activity coefficients are responsible for influencing the magnitude of various equilibrium constants of interest to chemistry. Solute activities have minimal influence in TEQA for two reasons:

1. Neutral molecules dissolved in water do not affect ionic strength.
2. Very dilute aqueous solutions are most likely found.

However, one aspect of TEQA that is strongly influenced by ionic strength and hence provides an opportunity for activity coefficients to play a role is the concept of salting out. The solubility of one chemical substance in another, like LLE, is also governed by the need for the substance to lower its Gibbs free energy by dissolving in a solvent. Isopropyl alcohol (IPA) or 2-propanol is infinitely soluble in water, as is true for most lower-molecular-weight alcohols. However, for a solution that might consist of

^{*}The concept of activity and activity coefficients are found in most physical and analytical chemistry texts that consider ionic equilibria. The texts listed in Ref. 4 are part of the author's personal library.

50% IPA and 50% water, the alcohol can be separated out as a separate phase if enough NaCl is added to almost saturate the system. This is a direct influence of ionic strength in an extreme case. The fact that polar solvents can be separated as an immiscible phase opens up new sample preparation opportunities. For example, Loconto and co-workers recently demonstrated that the homologous series of polar 2-aminoethanols could be efficiently partitioned into IPA from an aqueous sample of interest to wood chemists (5). The sample was saturated with NaCl, then extracted using IPA.

Two important relationships must be discussed that relate activity coefficients to ionic strength. Ionic equilibria are influenced by the presence of all ions in an aqueous solution. The most useful indicator of the total concentration of ions in a solution is the ionic strength, I . The ionic strength can be calculated if the concentration C_i of an ion whose charge is z_i is known according to

$$I = \frac{1}{2} \sum_i c_i z_i^2 \quad (3.5)$$

The summation is extended over all ions in solution. For example, consider two aqueous solutions, one containing 0.01M NaCl and the other one containing 0.01M K₂SO₄. Using Eq. (3.5), the ionic strength for the former solution is calculated to be 0.01M and that for the latter is 0.03M. Assume that a solution is created that consists of 0.01M in each salt. The ionic strength of such a mixture is calculated according to Eq. (3.5) to be 0.04M.

A knowledge of a solution's ionic strength enables a determination of the activity coefficient to be made. This can occur through the application of the Debye–Huckel equation according to

$$\log \gamma = \frac{-0.51z^2\sqrt{I}}{1 + \alpha\sqrt{I}/305}$$

where α refers to the size of the hydrated radius of the ion and z is the charge of the ion. This equation gives good approximations for ionic strengths below or equal to 0.1M. For ionic strengths less than 0.01M, the following relationship suffices:

$$\log \gamma = 0.51z^2\sqrt{I}$$

4. CAN THE DIFFERENCE BETWEEN K^0 VALUES FOR NaCl AND HOAc BE SHOWN GRAPHICALLY?

The thermodynamic relationship between standard state chemical potential differences and the position of chemical equilibrium can be shown graphically. Figure 3.2 illustrates what happens to the Gibbs free energy G when the solute is partitioned between an aqueous phase in contact with an immiscible organic phase, diethyl ether in this example. The hypothetical plots of G versus the mole fraction, denoted by X_i , of solute i dissolved in the ether phase are superimposed for comparison. When there is no solute in the ether phase, a standard state chemical potential, μ_{aq}^0 , can be realized. In the other extreme, when 100% of all of the mass of solute is in the ether phase

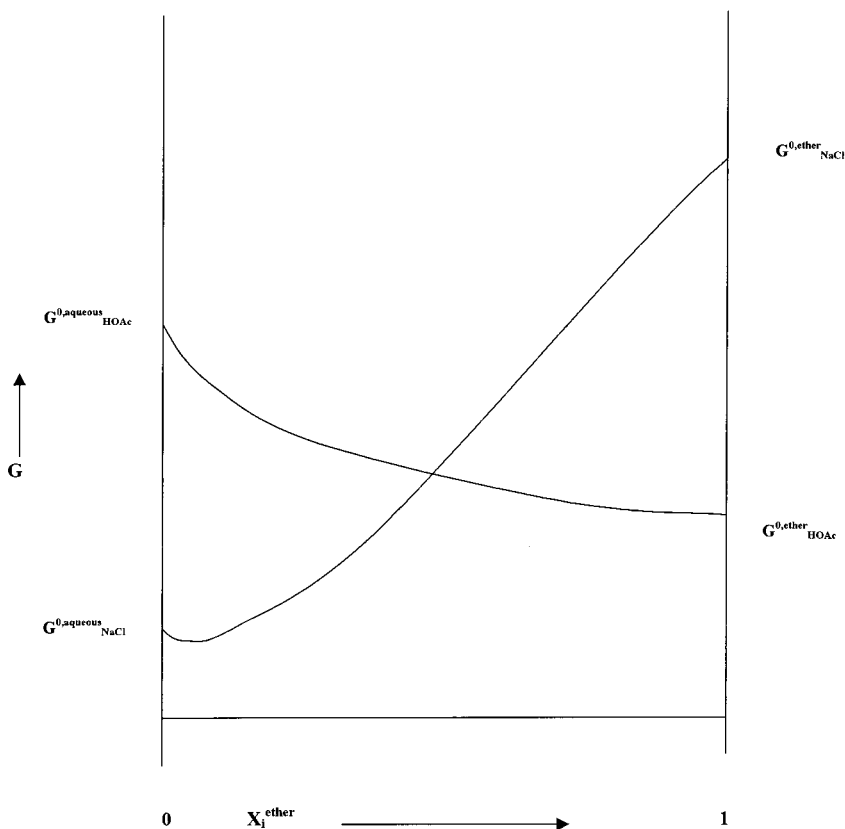


FIGURE 3.2 Hypothetical plot of free energy versus the mole fraction of solute in ether. $X_i^{\text{ether}} = n_i^{\text{ether}} / n_{\text{total}}$; $n_{\text{total}} = n_i^{\text{aqueous}} + n_i^{\text{ether}}$.

(i.e., having a mole fraction $X_{\text{ether}} = 1$), a standard state chemical potential, μ_{ether}^0 , can also be defined. The situation at $X_{\text{ether}} = 1$ is a hypothetical one in that for some solutes, 1 mol of solute cannot dissolve to that extent in an organic solvent like ether. This is particularly true for an ionically bonded substance such as sodium chloride. Imagine if this much NaCl could dissolve in ether! The free energy that would be required to dissolve as much as 1 mol of NaCl in 1 L of ether would be expected to be extremely large indeed!

Such is not the case when considering the free energy required for the dissolution of 1 mol of HOAc in 1 L of ether. The mole fraction of solute partitioned into the ether at equilibrium is that point along the x axis where the G is at a minimum or, in other words, the slope of the tangent line (i.e., dG/dX_i) is zero. It becomes quite evident when viewing this graphical display that the magnitude of standard state Gibbs free energies are chiefly responsible for the position along the x axis where G reaches a minimum. At this position, the mole fraction of each solute becomes fixed as defined by Eq. (3.3). Figure 3.2 shows that the Gibbs free energy is minimized at equilibrium for NaCl at a much lower mole fraction when compared to the value of the mole fraction for HOAc where its Gibbs free energy is minimized. In other words, the value of X_i where dG/dX_i is minimized at equilibrium depends entirely on the nature of the chemical compound. If a third solute is added to the original aqueous solution as depicted in Figure 3.1, it too would exhibit its own G -as-a-function-of- X_i plot and reach a minimum at some other point along the x axis. These concepts render Eq. (3.3) a bit more meaningful when graphically represented.

5. CAN WE RELATE K^0 TO ANALYTICALLY MEASURABLE QUANTITIES?

It becomes important to TEQA to relate the thermodynamic distribution constant, K^0 , to measurable concentrations of dissolved solute in both phases. Because the chemical potential for a given solute must be the same in both immiscible phases that are in equilibrium, Eq. (3.2) can be rewritten in terms of activity coefficients and concentration according to

$$\mu_{\text{ether}}^0 + RT \ln C_{\text{ether}} + RT \ln \gamma_{\text{ether}} = \mu_{\text{aq}}^0 + RT \ln C_{\text{aq}} + RT \ln \lambda_{\text{aq}}$$

Upon rearranging and simplifying, we get

$$RT \ln \frac{C_{\text{ether}}}{C_{\text{aq}}} + RT \ln \frac{\gamma_{\text{ether}}}{\lambda_{\text{aq}}} = -\Delta\mu^0$$

This equation can be solved for the ratio of measurable concentrations of solute in the ether phase to that of the water phase and this is shown by:

$$\frac{C_{\text{ether}}}{C_{\text{aq}}} = \frac{\gamma_{\text{ether}}}{\gamma_{\text{aq}}} e^{-\Delta\mu^0/RT} \quad (3.6)$$

If we define a partition constant K_D as a ratio of measurable concentrations of solute in both phases, we get

$$K_D \equiv \frac{C_{\text{ether}}}{C_{\text{aq}}} \quad (3.7)$$

Upon substituting Eq. (3.6) into Eq. (3.7), we obtain the relationship between the partition ratio and the thermodynamic distribution constant according to

$$K_D = \frac{\gamma_{\text{ether}}}{\gamma_{\text{aq}}} K^0 \quad (3.8)$$

Equation (3.8) is the desired outcome. In many cases with respect to TEQA, the activity coefficients of solutes in both phases are quite close to unity. The partition ratio and thermodynamic distribution constant can be used interchangeably.

For either NaCl or for HOAc or for any other solute distributed between immiscible liquids at a fixed temperature and pressure, provided that the concentration of solute is low (i.e., for the dilute solution case), K^0 can be set equal to the partition constant K_D because activity coefficients can be set equal to 1. The partition constant or Nernst distribution constant in our illustration for acetic acid partitioned between ether and water can be defined as

$$K_D = \frac{[\text{HOAc}]_{\text{ether}}}{[\text{HOAc}]_{\text{aq}}}$$

From the analytical results for measuring the concentration of HOAc in each phase introduced earlier, K_D can be calculated:

$$5 \times 10^{-2} M / 5 \times 10^{-2} M = 1$$

Likewise, from the analytical results for measuring the concentration of NaCl in each phase introduced earlier, K_D can be calculated:

$$1 \times 10^{-1} M / 1 \times 10^{-11} M = 1 \times 10^{-10}$$

6. IS LLE A USEFUL CLEANUP TECHNIQUE?

Two examples of how LLE is used not only to isolate the analyte of interest from possible interferences from the sample matrix but also to provide an important “cleanup” are now discussed. Both procedures which were then incorporated into respective methods yield an extract that is ideally free of interferences that can then be used in the determinative step to quantitate the presence of analyte that was originally in the sample.

In the first case, an environmental sample that contains a high concentration of dissolved inorganic salts such as NaCl is suspected of containing trifluoroacetic acid (TFA). TFA is a known by-product from the recently understood persistence of fluorocarbons in the environment (6). The physical and chemical properties of TFA are well known. When dissolved in water, TFA is a moderately strong carboxylic acid with a pK_a lower than that of acetic acid. TFA also has an infinite solubility in water. TFA is not directly amenable to detection by GC because it cannot be sufficiently vaporized in the hot injection port of the GC. It is not good practice to make a direct aqueous injection into a GC that possesses a column which contains the commonly used silicone polymer as liquid phase. Hence, it is necessary to prepare an analytical reference standard in such a way that (a) TFA can be made amenable to analysis by GC and (b) extracts that contain TFA must be nonaqueous. TFA could be determined by a direct aqueous injection if a different instrumental technique is used. The options here include either high-performance liquid chromatography (HPLC) in one of its several forms, ion chromatography (IC), or capillary electrophoresis (CE). There is a gain, however, if a sample preparation technique can be developed which concentrates the sample. Seiber's group took the following approach to the determination of TFA in environmental waters (7).

The highly salted aqueous sample that is expected to contain the targeted analyte TFA is initially acidified to suppress the ionization of the acid according to



where the subscript (aq) refers to the fact that each ionic species is dissolved in water and is surrounded by water dipoles. The double arrow denotes that when TFA is initially dissolved in water, a dynamic chemical equilibrium is quickly established whereby hydronium and trifluoroacetate ions exist in water along with undissociated TFA. Upon acidifying, the extent of this ionization is suppressed and a new equilibrium concentration of hydronium, trifluoroacetate, and TFA is reestablished with significantly higher concen-

trations of TFA and hydronium ion and much lower trifluoroacetate. Refer to any number of texts which elaborate on the principles of ionic equilibrium that govern the extent of acid dissociation (8).*

The acidified aqueous environmental water sample is then extracted with a nonpolar solvent such as hexane, iso-octane, dichloromethane (methylene chloride), or some other common water-immiscible solvent. TFA is partitioned into the extractant to an appreciable extent owing to the fact that its ionization has been suppressed in the aqueous phase and the trifluoromethyl moiety gives a hydrophobic character to the molecule. The inorganic salts are left behind in the aqueous phase. Upon physically separating the phases and placing the organic phase in contact with a second aqueous phase that has been made alkaline or basic by the addition of NaOH or KOH, TFA molecules diffuse throughout the bulk of the extractant toward the interfacial surface area where they are ionized according to



After the rate of TFA transport through to the interface from the bulk extractant and into the alkaline aqueous phase becomes equal to the rate of TFA from the bulk alkaline aqueous phase through to the extractant and equilibrium reestablished, a new partitioning has occurred with most of the original TFA now in the alkaline aqueous phase. The cleanup has been accomplished because the aqueous phase contains TFA, as its conjugate base, without any dissolved inorganic salts. The alkaline aqueous matrix is then passed through a disk which contains anion-exchange sites whereby trifluoroacetate can be retained by the ion-exchange interaction. The disk is then placed into a 22-mL headspace vial containing 10% sulfuric acid in methanol and the vial sealed tightly. Heating at 50°C for a finite period of time converts TFA to its methyl ester. The headspace, which now contains methyl trifluoroacetate, is sampled with a gas-tight GC syringe and injected into a GC. The headspace technique eliminates any solvent interference.

The second case taken from the author's own work uses LLE to initially clean up an aqueous sample taken from the environment that might contain, in addition to the analyte of interest, other organic compounds that may interfere in the determinative step (9). The analytes of interest are the class of chlorophenoxy herbicides (CPHs) and includes 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2,4,5-trichlorophenoxypropionic acid (Silvex). CPHs are

*In addition to the reference sources cited in Ref. 4, a number of texts on water chemistry discuss ionic equilibria and a recent book are listed in Ref. 8.

used as herbicides in agricultural weed control, and because of this, CPHs are routinely monitored in drinking water supplies. CPHs are usually produced as their corresponding amine salts or as esters. An initial alkaline hydrolysis of the sample is needed to convert the more complex forms to their corresponding conjugate bases. The sample is then extracted using a nonpolar solvent. This LLE step removes possible organic interferences while leaving the conjugate bases to the CPHs in the aqueous phase. A cleaned-up alkaline aqueous phase results which can now be acidified and either reextracted (LLE) or passed through a chemically bonded solid sorbent to isolate the CPHs and possibly achieve a concentration of the CPHs from that in the original sample. As was true in the first case, the ionizable properties of these analytes can be exploited to yield a clean extract that can be quantitatively determined. Between 95% and 100% recoveries for the three CPHs cited were obtained from water spiked with each CPH. No influence of these high-percentage recoveries upon inserting an initial LLE step was observed (9).

In contrast, the more conventional approach to trace CPH residue analysis serves to illustrate this difference in approaches to sample preparation. A water sample taken from the environment is initially acidified to preserve its chemical composition prior to sample preparation and analysis. At the onset of sample preparation, the water sample is made alkaline. To this alkaline aqueous phase, nonpolar solvent is added and the immiscible phases are shaken in a glass separatory funnel. Esters of CPHs, being nonpolar themselves, obey the universal principle that “like dissolves like” and partition into the organic phase. The free CPH acids remain in the aqueous phase. If only the formulated esters of CPHs are of interest, the extract can be cleaned up and analyzed as the flow scheme shows. However, if it is desirable to convert the esters to acids, as is the case in most regulatory methods, a base hydrolysis is conducted on the organic phase that converts these CPH esters to their corresponding salts. The aqueous phase is reacidified and a second LLE is performed. The extracted CPHs are derivatized and converted to their corresponding methyl esters using any of the more common derivatization reagents. Following a cleanup step, the extract is ready for injection into a GC with a chlorine-selective detector such as an electron-capture detector (ECD) or an electrolytic conductivity detector (EICD). This approach to sample preparation is a good example of the complexity involved in many of the methods of TEQA. If 1 L of an environmental water sample is taken through this method, it is likely that a concentration of 10 ppb of 2,4-D originally present in the sample can be separated from other CPHs, identified, detected, and quantified using all of the techniques available in TEQA.

These two examples clearly demonstrate the importance of secondary equilibria phenomena, particularly when the analyte of interest is ionizable in an environmental aqueous sample such as groundwater. Both examples exploit secondary equilibria in developing alternative methods that include LLE in extraction and in cleanup when applied to the complex sample matrices commonly encountered in TEQA. In the next section, the mathematical framework that underlies secondary equilibria will be presented.

7. HOW DO WE ACCOUNT FOR SECONDARY EQUILIBRIA IN LLE?

Let us return to the ether/aqueous immiscible distribution equilibrium model introduced earlier, refer to Figure 3.1. What if the aqueous solution, prior to adding any ether, was made alkaline by the addition of NaOH? We know that the chloride ion concentration in the original aqueous solution would not change, but what about the HOAc? We also know that acetic acid is a weak acid and undergoes dissociation to hydronium ions and acetate ions. The extent of this dissociation is governed by the acid dissociation constant, K_a . The double arrow notation is used in the following reaction to show that prior to the addition of hydroxide ion to an aqueous solution that contains dissolved acetic acid, the ionic equilibrium is already established.



The effect of the added hydroxide ion is to shift the position of equilibrium to favor the product acetate and, thus, to remove HOAc from the aqueous phase. HOAc molecules in the ether phase partition back to the aqueous phase until chemical potentials become equivalent and the magnitude of K_D is restored to the same value that the system had before the addition of the hydroxide ion.

Does this pH adjustment have any effect on the partitioning of HOAc between immiscible phases? By definition, only neutral HOAc can partition between phases. The value for the partition ratio must be preserved based on the thermodynamic arguments put forth earlier. This must mean that the concentrations of HOAc in the ether phase must be reduced due to the pH adjustment because the concentration of undissociated HOAc in the aqueous phase has also been reduced. This is illustrated for the HOAc, only, in Figure 3.3. Our model assumes that the only chemical form of acetic acid in the ether phase is HOAc and that only acid dissociation of HOAc occurs in the aqueous phase. Because K_D accounts only for undissociated forms of

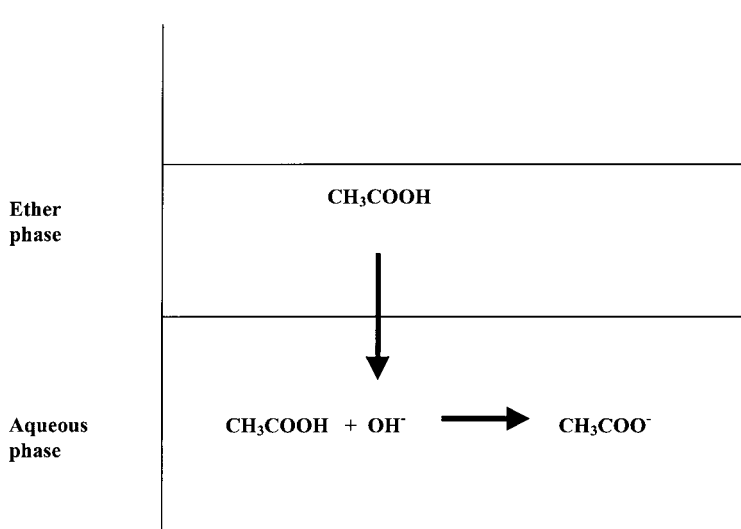


FIGURE 3.3 Distribution of HOAc between two immiscible solvents.

acetic acid, a new constant is needed to completely account for the undissociated acetic ion and for the acetate ion. This constant is called the distribution ratio, D , and is defined according to

$$D \equiv \frac{\sum_j [\text{A}]_o}{\sum_k [\text{A}]_{\text{aq}}} \quad (3.9)$$

where $[\text{A}]_o$ refers to the concentration or activity of the j th chemical species in the organic or extractant phase and $[\text{A}]_{\text{aq}}$ refers to the concentration or activity of the k th chemical species in the aqueous phase.

The magnitude of D enables one to understand the extent to which all chemical forms of the analyte of interest are partitioned between two immiscible phases. D accounts for all secondary equilibrium effects that occur. Let us go back to the concept of acetic acid partitioning between diethyl ether and water while considering the influence of the secondary equilibrium, that of weak acid dissociation due to an adjustment of the pH of the aqueous phase. This discussion will help us enlarge the scope of LLE and set the stage for further insights into the role of secondary equilibrium.

We start by using Eq. (3.9) to define the different chemical species that are assumed to be present and then we will proceed to substitute secondary equilibrium expressions governed by acid/base dissociation constants or

metal chelate formation constants. In the case of HOAc that is partitioned between ether and water, let us assume that only monomeric forms of HOAc exist in both phases and define the distribution ratio, D , according to

$$D = \frac{[\text{HOAc}]_{\text{ether}}}{[\text{HOAc}]_{\text{aq}} + [\text{OAc}^-]_{\text{aq}}} \quad (3.10)$$

Acetic acid dissociates in pure water to the extent determined by the magnitude of the acid dissociation constant, K_a . Based on the Law of Mass Action, K_a is defined as

$$K_a = \frac{[\text{H}^+][\text{OAc}^-]}{[\text{HOAc}]} \quad (3.11)$$

Let us solve Eq. (3.11) for the acetate ion concentration that is in equilibrium with the hydronium ion, H^+ , and undissociated HOAc:

$$[\text{OAc}^-] = K_a \frac{[\text{HOAc}]}{[\text{H}^+]}$$

Substituting for $[\text{OAc}^-]$ in Eq. (3.10) and simplifying yields a fruitful relationship:

$$D = \frac{[\text{HOAc}]_{\text{ether}}}{[\text{HOAc}]_{\text{aq}} + K_a \frac{[\text{HOAc}]_{\text{aq}}}{[\text{H}^+]}}$$

This expression can be further rearranged by factoring out the ratio of both molecular forms of HOAc:

$$D = \frac{[\text{HOAc}]_{\text{ether}}}{[\text{HOAc}]_{\text{aq}}} \left[\frac{1}{1 + K_a/[\text{H}^+]}\right]$$

This gives an expression for D in terms of a ratio of concentrations in both phases for the undissociated acid forms, which is exactly our definition of the distribution constant for the partitioning of HOAc between ether and water. Expressing D in terms of K_D yields an important relationship:

$$D = K_D \left[\frac{1}{1 + K_a/[\text{H}^+]}\right] \quad (3.12)$$

Equation (3.12) clearly shows the dependence of the distribution ratio on the secondary equilibrium (i.e., the weak acid dissociation,) and on the extent of the primary equilibrium (i.e., the partitioning equilibrium of molecular HOAc between two immiscible phases). If Eq. (3.12) is rearranged, we get

$$D = \frac{K_D[\text{H}^+]}{K_a + [\text{H}^+]}$$

A plot of D versus $[\text{H}^+]$ is shown in Figure 3.4. The graph is hyperbolic, and upon careful examination, it would appear to resemble the Michaelis–Menten enzymes kinetics found in biochemistry (10). The plot in Figure 3.4 as well as Eq. (3.12) show that in the limit as the hydronium ion concentration gets very large, K_a becomes small in comparison to $[\text{H}^+]$, and in the limit of a very large hydronium ion concentration, the following can be stated: In the limit as

$$[\text{H}^+] \rightarrow \infty$$

it is evident that

$$D \rightarrow K_D$$

The partition constant, K_D , and the acid dissociation constant, K_a , for acetic acid can be found experimentally from a plot of D versus $[\text{H}^+]$, as shown in Figure 3.4. Let $D = \frac{1}{2}K_D$ in Eq. (3.10) so that

$$\frac{1}{2}K_D = K_D \left[\frac{[\text{H}^+]}{K_a + [\text{H}^+]} \right]$$

Eliminating K_D and solving this equation for K_a gives

$$K_a = [\text{H}^+]$$

Hence, the acid dissociation constant for HOAc could be calculated. One would need to know experimentally exactly how D varies with the concentration of hydronium ion for this LLE in order to prepare a precise plot. It becomes difficult to estimate K_D from the hyperbolic curve shown in Figure 3.4. Equation (3.13) can be rearranged by taking reciprocals of both sides and rewriting Eq. (3.13) in the form of an equation for a straight line of form $y = mx + b$, where m is the slope and b is the y intercept:

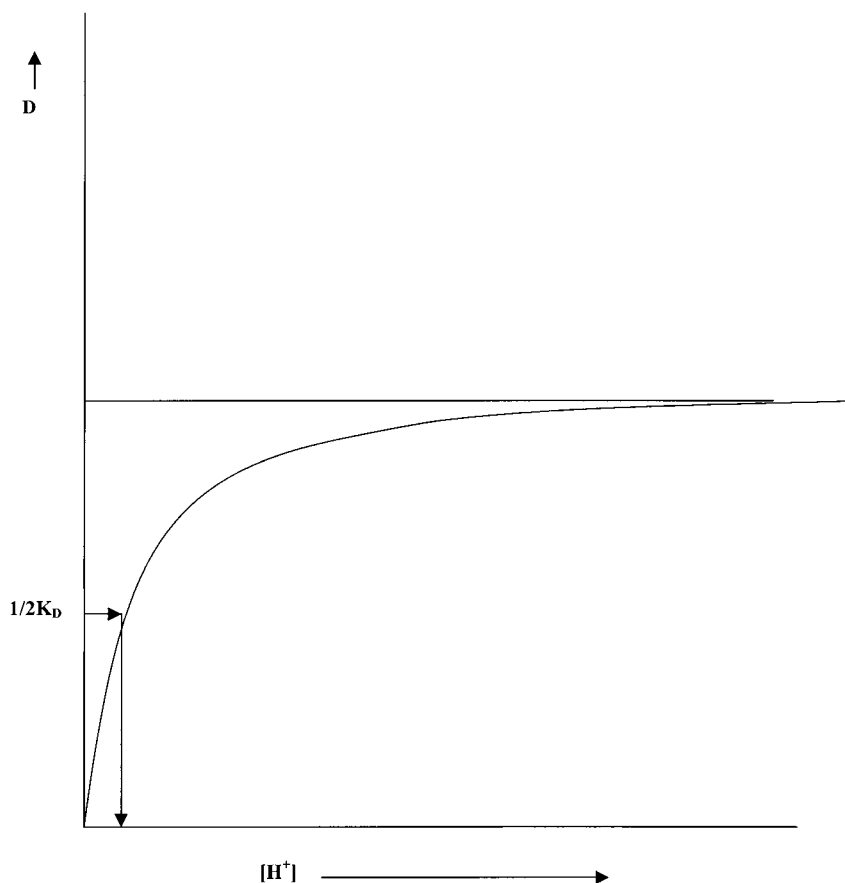


FIGURE 3.4 Plot of the distribution ratio versus $[H^+]$.

$$\frac{1}{D} = \left(\frac{K_a}{K_D} \right) \frac{1}{[H^+]} + \frac{1}{K_D}$$

A plot of $1/D$ versus $1/[H^+]$ yields a straight line whose slope m is equal to the ratio K_a/K_D and the y intercept b is equal to $1/K_D$. In this manner, both equilibrium constants can be determined with good precision and accuracy (10).

Alternatively, Eq. (3.12) can be viewed in terms of the primary equilibrium as represented by K_D and in terms of secondary equilibrium as represented by α_{HOAc} . Let us define α_{HOAc} as the fraction of neutral or undissociated HOAc present according to

$$\alpha_{\text{HOAc}} = \frac{[\text{HOAc}]_{\text{aq}}}{[\text{HOAc}]_{\text{aq}} + [\text{OAc}^-]_{\text{aq}}}$$

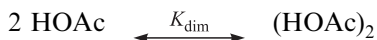
Upon simplifying, it can be shown that Eq. (3.12) can be rewritten as

$$D = K_D \alpha_{\text{HOAc}}$$

Upon examination of this relationship among D , K_D , and α_{HOAc} , it becomes evident that the distribution ratio depends on the extent to which a solute (in our example, acetic acid), distributes itself between two immiscible phases (e.g., ether and water). At the same time, this solute is capable of exhibiting a secondary equilibrium (i.e., that of acid dissociation in the aqueous phase), as determined by the fraction of all acetic acid that remains neutral or undissociated. We will introduce this concept of fractional dissociation as just defined when we discuss LLE involving the chelation of transition metal ions from an aqueous phase to a water-immiscible organic phase.

8. WHAT IF THE CHEMICAL FORM OF HOAc CHANGES IN THE ORGANIC PHASE?

The above formalism assumed that only the monomeric form of HOAc exists in the ether phase. Carboxylic acids are known to dimerize in organic solvents that have a low dielectric constant. Let us assume we have acetic acid forming a dimer in the organic phase. This tendency may be more prominent if HOAc is dissolved in a nonpolar solvent like hexane as compared to a moderately polar solvent like diethyl ether. The formation of a dimer can be depicted by:



The extent to which the dimer is favored over that of the monomer is determined by the magnitude of K_{dim} . This added secondary equilibrium, this time appearing in the organic phase, is shown in Figure 3.5. The fundamental basis for the partitioning of HOAc between ether and water as introduced by the Nernst law is not violated and still is given by K_D . The measurable concentrations $[\text{HOAc}]_{\text{ether}}$ and $[\text{HOAc}]_{\text{aq}}$ will definitely differ with this added dimerization reaction. Let us define D for this distribution equilibrium involving weak acid dissociation of HOAc in the aqueous phase and, at the same time, dimerization of HOAc in the ether phase as follows:

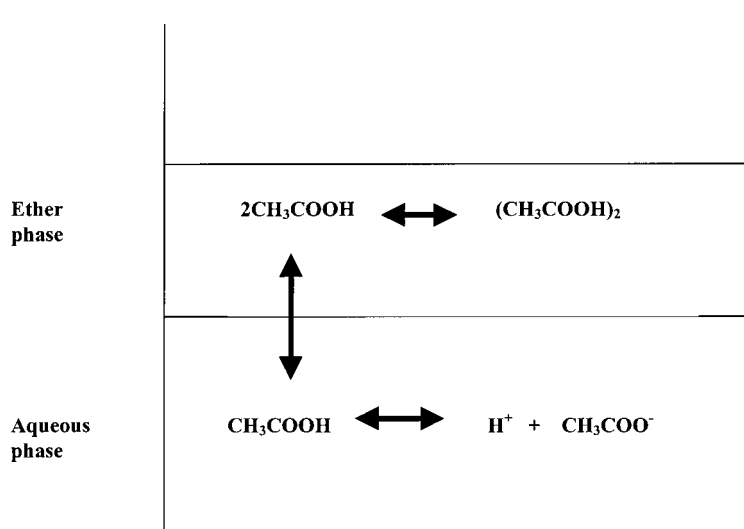


FIGURE 3.5 Distribution of HOAc between ether and water assuming dimerization in ether phase.

$$D = \frac{[\text{HOAc}]_{\text{ether}} + 2[(\text{HOAc})_2]_{\text{ether}}}{[\text{HOAc}]_{\text{aq}} + [\text{OAc}^-]_{\text{aq}}} \quad (3.14)$$

The following expressions are applicable to this distribution equilibrium and are defined as follows:

$$K_D = \frac{[\text{HOAc}]_{\text{ether}}}{[\text{HOAc}]_{\text{aq}}}$$

$$K_{\text{dim}} = \frac{[(\text{HOAc})_2]_{\text{ether}}}{[\text{HOAc}]_{\text{ether}}^2}$$

$$K_a = \frac{[\text{H}^+]_{\text{aq}}[\text{OAc}^-]_{\text{aq}}}{[\text{HOAc}]_{\text{aq}}}$$

Upon substituting the above three definitions into Eq. (3.14) and simplifying yields the following relationship:

$$D = \frac{K_D \{1 + 2K_{\text{dim}}[\text{HOAc}]_{\text{ether}}\}}{1 + K_a/[\text{H}^+]_{\text{aq}}} \quad (3.15)$$

Equation (3.15) shows that the value of the distribution ratio, D , depends not only on the equilibrium constants as indicated and the pH but also on the concentration of HOAc in the ether phase.

It becomes instructive to compare Eqs. (3.12) and (3.15). The influence of dimerization in the organic phase results in an additional term in the numerator for the distribution ratio, D . This additional term depends on the magnitude of K_{dim} and on the concentration of HOAc in this phase. In the case of HOAc, values for K_{dim} range from a high of 167 for benzene as the solvent to a low of 0.36 for diethyl ether as the solvent (11). The larger the value for K_{dim} , the larger is the magnitude of D , and as, we shall see in the next section, the higher is the percent recovery.

9. IF WE KNOW D , CAN WE FIND THE % RECOVERY?

The discussion so far has focused on first establishing the validity of the partition constant, K_D , for LLE and then extending this to the distribution ratio, D . We have shown that setting up expressions involving D become more useful when secondary equilibria exists. Before we consider other types of secondary equilibria, the importance of knowing how D relates to the percent recovery, % E , will be discussed. Percent recovery is an important QC parameter when LLE, SPE, and SFE techniques are used. Most EPA methods discussed in Chapter 1 require that the % E be measured for selected analytes in the same matrix as that for samples. This is particularly important as applied to the EPA methods for trace organics. In Chapter 2, we showed how % E is used in the statistical treatment of experimental data.

The determination of % E is paramount in importance toward establishing an alternative method in TEQA. A method that isolates phenol from wastewater samples using LLE and yields a consistently high % E is preferable to an alternative method that yields a low and inconsistent % E . As we showed in Chapter 2, a high % E leads to lower method detection limits (MDLs). However, if the alternative method significantly reduces sample preparation time, then a trade-off must be taken into account. Lower MDLs versus a long sample prep time! A practical question naturally arises here. What does the client want and what degree of trade-off is the client willing to accept?

Let C_0 represent the concentration of a particular analyte of interest after being extracted into an organic solvent whose volume is V_0 from an aqueous sample whose volume is V_{aq} . Assume also that the concentration of analyte that remains in the aqueous phase after extraction is C_{aq} . Let us define the fraction of analyte extracted E by

$$E \equiv \frac{\text{amt}_o}{\text{amt}(\text{total})}$$

where amt_o refers to the amount of analyte extracted into the organic phase and $\text{amt}(\text{total})$ refers to the total amount of analyte originally present in the aqueous sample. The fraction extracted can be expressed as follows:

$$E = \frac{C_o V_o}{C_o V_o + C_{\text{aq}} V_{\text{aq}}} = \frac{D\beta}{1 + D\beta} \quad (3.16)$$

where β is defined as the ratio of the volume of the organic phase, V_o , to the volume of the aqueous phase, V_{aq} according to

$$\beta = V_o / V_{\text{aq}}$$

The percent recovery is obtained from the fraction extracted, E , according to

$$\% \text{ Recovery} = E \times 100$$

Equation (3.16) shows that the fraction extracted and hence the percent recovery depend on two factors. The first is the magnitude of the distribution ratio which is dependent on the physical/chemical nature of each analyte and on the chemical nature of the extractant. The second factor is the phase ratio β . The magnitude is usually fixed if the extractant is not changed, whereas the phase ratio can be varied. If, instead of a single batch LLE, a second and third successive LLE is carried out on the same aqueous solution by removing the extractant and adding fresh solvent. After allowing time for partition equilibrium to be attained, while keeping the phase ratio constant, it can be shown that the total fraction now extracted is $E(1 - E)$. If a third successive extraction is performed while maintaining the same phase ratio, $E(1 - E)^2$ will be the total fraction of analyte extracted. The fraction remaining in the aqueous phase following n successive LLEs is $(1 - E)^{n-1}$. To achieve at least a 99% recovery, Eq. (3.16) suggests that the product βD must be equal to or greater than 100. Even with a product $\beta D = 10$, two successive LLEs will remove 99% of the amount of analyte originally in an aqueous environmental sample (12).

10. ARE ORGANICS THE ONLY ANALYTES THAT WILL EXTRACT?

Our examples so far have focused on neutral organic molecules such as acetic acid. The majority of priority pollutant organics of importance to TEQA are neutral molecules in water whose pH is within the 5–8 range. Before we leave the principles that underlie LLE, the answer to the question just posed is yes! Consider the significant difference in K_D versus HOAc partition constants discussed earlier. Ionic compounds have little to no tendency to partition into a moderate to nonpolar organic solvent. If, however, an ion can be converted to a neutral molecule via chemical change, this ion can exhibit a favorable K_D . This is accomplished in two ways: chelation of metal ions and formation of ionpairs. The mathematical development of a metal chelate is discussed in this section.

A number of organic chelating reagents exist that coordinate various metal ions, and the metal chelate that results consists of neutral molecules. This neutral or uncharged metal chelate will have a K_D much greater than 1. Metal ions initially dissolved in an aqueous phase such as a groundwater sample can be effectively removed by metal chelation LLE. Commonly used chelating reagents include four-membered bidentate organic compounds such as dialkyldithiocarbamates, five-membered bidentates such as 8-hydroxyquinoline and diphenylthiocarbazone, dithizone, and polydentates such as pyridylazonaphthol. 8-Hydroxyquinoline, commonly called oxine, HOx, is the chelating reagent used in this section to introduce the mathematical relationships for metal chelation LLE. Similar equations can be derived for other chelating reagents.

Figure 3.6 depicts the principal primary and secondary equilibria that would be present if oxine is initially dissolved in an appropriate organic solvent that happens to be less dense than water. If this solution is added to an aqueous solution that contains a metal ion such as copper(II) or Cu^{2+} , two immiscible liquid phases persist. The copper(II) oxinate that initially forms in the aqueous phase, oxine, itself is an amphiprotic weak acid that quickly partitions into the organic phase. Being amphiprotic means that oxine itself can accept a proton from an acid and can also donate one to a base. The degree to which oxine either accepts or donates a proton is governed by the pH of the aqueous solution. The acidic property is the only one considered in the development of the equations considered below. The formation of a Cu oxine chelate can proceed via 1:1 and 1:2 stoichiometry. The fact that it is the 1:2 chelate that is neutral and, therefore, is the dominant form that partitions into the nonpolar solvent is important. All of the competing primary and secondary equilibria can be

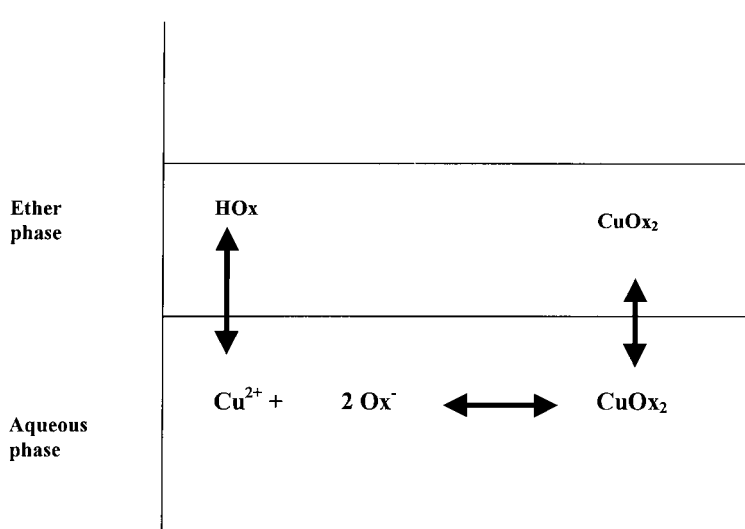


FIGURE 3.6 Distribution of copper oxinate between ether and water.

combined to yield a relationship that enables the distribution ratio to be defined in terms of measurable quantities.

The distribution ratio, D , for the immiscible phases and equilibria shown in Figure 3.6 is first defined as the ratio of chelated copper in the organic phase to the concentration of free and chelated copper in the aqueous phase. Expressed mathematically,

$$D \equiv \frac{[\text{CuOx}_2]_o}{[\text{Cu}^{2+}]_{\text{aq}} + [\text{CuOx}_2]_{\text{aq}}}$$

Similar to what was done earlier for HOAc , we can define α_{Cu} as the fraction of free Cu^{2+} in the aqueous phase: then,

$$\alpha_{\text{Cu}} = \frac{[\text{Cu}^{2+}]_{\text{aq}}}{[\text{Cu}^{2+}] + [\text{CuOx}_2]_{\text{aq}}}$$

so that

$$D = \frac{[\text{CuOx}_2]_o}{[\text{Cu}^{2+}]/\alpha_{\text{Cu}}} \quad (3.17)$$

Use of α_{Cu} is a simple and convenient way to account for all of the many side reactions involving the metal ion. Substituting the equilibrium expressions into Eq. (3.17) yields

$$D = \frac{K_D^{\text{CuOx}_2} \beta_2 K_a^2 [\text{HOx}]_o^2}{K_D^{\text{HOx}} [\text{H}^+]_{\text{aq}}} \alpha_{\text{Cu}} \quad (3.18)$$

We have assumed that the protonation of HOx as discussed earlier is negligible. Equation (3.18) states that the distribution ratio for the metal ion chelate LLE depends on the pH of the aqueous phase and on the ligand concentration. $K_D^{\text{HOx}_2}$, β_2 , and α are dependent on the particular metal ion. This enables the pH of the aqueous phase to be adjusted such that a selected LLE can occur. One example of this selectivity is the adjustment of the pH to 5 and extraction as their dithizonates to selectively separate Cu^{2+} from Pb^{2+} and Zn^{2+} (13).

The metal chelate LLE was much more common 25 years ago when it was the principal means to isolate and recover metal ions from aqueous samples of environmental interest. The complexes were quantitated using a visible spectrophotometer because most complexes were colored. A large literature exists on this subject (14). The technological advances in both atomic absorption and inductively coupled plasma–atomic emission spectroscopy have significantly reduced the importance of metal chelate LLE to TEQA. However, metal chelate LLE becomes important in processes whereby selected metal ions can be easily removed from the aqueous phase.

11. CAN ORGANIC CATIONS OR ANIONS BE EXTRACTED?

We have discussed the partitioning of neutral organic molecules from an aqueous phase to a nonpolar organic solvent phase. We have discussed the partitioning of metal ions once they have been converted to neutral metal chelates. In this section, we discuss the partitioning of charged organic cations or charged organic anions. This type of LLE is termed ionpairing. Ion pair LLE is particularly relevant to TEQA, as will be shown below. We start by using equilibrium principles and assume that the only equilibria are the primary ones involving the partitioning of the ion pair between an aqueous phase and a lighter-than-water organic phase. The secondary equilibria consist of formation of the ion pair in the aqueous phase. Also, all cations and anions are assumed not to behave as weak acids or bases. For the formation of the ion pair in the aqueous phase, we have



The ion pair CA , once formed, is then partitioned into an organic solvent that is immiscible with water according to



The distribution ratio, D , with respect to the anion for IP-LLE is defined as

$$D_{A^{-}} = \frac{[CA]_{org}}{[CA]_{aq} + [A^{-}]_{aq}}$$

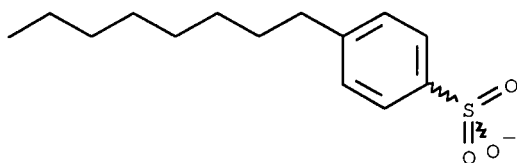
In a manner similar to that developed earlier, D can be rewritten as

$$D_{A^{-}} = K_D \left\{ \frac{K_{IP}[C^{+}]}{1 + K_{IP}[C^{+}]} \right\} \quad (3.19)$$

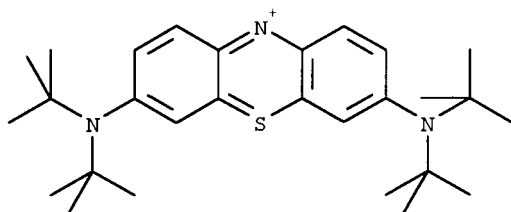
The distribution ratio is seen to depend on the partition coefficient of the ion pair, K_D , the extent to which the ion pair is formed, K_{IP} , and the concentration of the cation in the aqueous phase. Equation (3.19) shows some similarity to Eq. (3.13).

12. IS THERE AN IMPORTANT APPLICATION OF IP-LLE TO TEQA?

Equation (3.19) suggests that if an ion pair that exhibits a high partition coefficient, K_D , forms the ion pair to a great extent (i.e., has a large value for K_{IP}) β , then a large value for D enables an almost complete transfer of a particular anion to the organic phase. Of all the possible ion pair complexes that could form from anions that are present in an environmental sample, the isolation and recovery of anionic surfactants using methylene blue is the most commonly employed IP-LLE technique used in environmental testing labs today. The molecular structure of this ion pair formed from a large organic anion that is prevalent in wastewater such as an alkyl benzenesulfonate, a common synthetic detergent, using a large organic cation such as methylene blue is as follows:



alkylbenzenesulfonate



methylene blue

This ion pair absorbs visible light strongly at a wavelength of 652 nm. Because a method that might be developed around this ion pair and its high % recovery into a non-polar solvent (a most commonly used one is chloroform) is unselective, a cleanup step is usually introduced in addition to the initial LLE step. Of all possible anion surfactants, sodium salts of C_{10} – C_{20} do not form an ion pair with methylene blue, whereas anionic surfactants of the sulfonate and sulfate ester types do. Sulfonate-type surfactants contain sulfur covalently bonded to carbon, whereas the sulfate ester type of surfactant contains sulfur covalently bonded to oxygen, which, in turn, is covalently bonded to sulfur. A good resource on the analysis of surfactants in all of its forms including some good definitions was published earlier (15). The type of surfactants that form an ion pair and give rise to a high % recovery are termed “methylene blue active substance or MBAS.” A micro-scaled version to the conventional method (16) for the determination of MBAS in wastewater is introduced as one of the student experiments discussed in Chapter 5.

13. ARE THERE OTHER EXAMPLES OF NONSPECIFIC LLE PERTINENT TO TEQA?

In Chapter 1, the determination of total petroleum hydrocarbons (TPHs), was discussed in relation to EPA method classifications. This method is of widespread interest in environmental monitoring, particularly as this relates to the evaluation of groundwater or wastewater contamination. There are several specific determinations of individual chemical components related to

either gasoline, fuel oil, jet fuel, or lubricant oil that involve an initial LLE followed by a GC determinative step. Methods for these require LLE, possible cleanup followed by GC separation, and detection usually via a flame ionization detector FID. Specific methods are usually required when the type of petroleum hydrocarbon is of interest. There is almost equal interest among environmental contractors for a non specific, more universal determination of the petroleum content without regard to “chemical specificity.” A sample of groundwater is extracted using a nonpolar solvent. The extracted TPHs are then concentrated via evaporation either by use of a rotary evaporator, Kuderna-Danish evaporative concentrator, or via simple distillation to remove the extracting solvent. The residue that remains is usually a liquid and the weight of this oily residue is obtained gravimetrically. An instrumental technique that represents an alternative to gravimetric analysis involves the use of quantitative infrared (IR) absorption. If the extracting solvent lacks carbon-to-hydrogen covalent bonds in its structure, then the carbon-to-hydrogen stretching vibration could be used to quantitate the presence of TPHs. The most common solvent that emerged was 1,1,2-trichloro-trifluoroethane (TCTFE). With the eventual total phasing out of Freon-based solvents, the EPA has reverted back to the gravimetric determinative approach. It is not possible to measure trace concentrations of TPHs via quantitative IR using a hydrocarbon solvent due to the strong absorption due to the presence of carbon-to-hydrogen covalent bonds. This author maintains that labs could recycle and reuse the spent TCTFE without any release of this Freon-type solvent to the environment while preserving the quantitative IR determinative method. Only time and politics will determine which method will dominate in the future. Nevertheless, the technique of LLE to isolate and recover TPHs from water contaminated with oil remains important.

14. CAN LLE BE “DOWNSIZED”?

We now introduce some recently reported and interesting research that reinforces the basic concepts of LLE. Cantwell and co-workers have introduced the concept of a true LLE that has been “downsized” to a micro extraction scale (17). In the past, the concept of a micro-LLE, abbreviated μ LLE, as introduced by the EPA and promulgated through their 500 series of methods, was designed to conduct TEQA on samples from sources of drinking water. Method 508 required that 35 mL of groundwater or tap water be placed in a 40 mL vial and extracted with exactly 2 mL of hexane. Organochlorine pesticides such as aldrin, alachlor, dieldrin, heptachlor, and so forth are easily partitioned into the hexane. A 1- μ L aliquot is then injected either manually or via autosampler into a GC-ECD to achieve

the goal of TEQA. As long as emulsions are not produced, this “down-sized” version of LLE works fine. Wastewater samples are prone to emulsion formation and this factor limits the scope of samples that can be extracted by this mini-LLE technique.

Cantwell’s group has taken this scale down by a factor of about 20 to the 1 mL and below sample volume levels. Some interesting mathematical relationships that serve to reinforce the principles discussed earlier are introduced here. It does not matter whether an analyst uses a liter of groundwater sample, a milliliter, or even a microliter. The principles remain the same.

The principle of mass balance requires that the amount of a solute that is present in an aqueous sample (e.g., groundwater), $\text{amt}_{\text{initial}}$, remains mathematically equivalent to the sum of solute in both immiscible phases. Matter cannot escape, theoretically that is. If the initial amount of a solute is distributed between two immiscible phases, an organic phase, o , and an aqueous phase, aq , mass balance considerations require that

$$\text{amt}_{\text{initial}} = \text{amt}_{\text{aq}} + \text{amt}_o$$

We now seek to relate the concentration of solute that remains in the aqueous phase after μLLE to the original concentration of solute, C_{initial} . For example, a groundwater sample that contains dissolved organochlorine pesticides such as DDT can be mathematically related to the partitioned concentrations in both phases according to

$$C_{\text{initial}} = \frac{V_{\text{aq}}C_{\text{aq}} + V_oC_o}{V_{\text{aq}}}$$

We would like to express the concentration of analyte in the organic phase, C_o , in terms of the initial concentration of analyte that would be found in groundwater, C_{initial} . Upon dividing through by V_{aq} and eliminating C_{aq} because for LLE,

$$C_{\text{aq}} = \frac{C_o}{K_D}$$

gives

$$C_{\text{initial}} = \frac{V_{\text{aq}}\left[\frac{C_o}{K_D}\right] + V_oC_o}{V_{\text{aq}}}$$

Substituting for the phase ratio, β , dividing the numerator and denominator by V_{aq} , and rearranging gives

$$C_{\text{initial}} = C_o \left[\frac{1}{K_D} + \frac{\beta}{1} \right]$$

Rearranging and solving for C_o gives

$$C_o = C_{\text{initial}} \left[\frac{K_D}{1 + \beta K_D} \right] \quad (3.20)$$

Hence, the concentration of solute present in the organic phase can be directly related to the concentration of solute initially present in the aqueous groundwater sample, C_{initial} , provided the partition coefficient and phase ratio, β , are known. The reader should see some similarity between Eqs. (3.20) and (3.16). Equation (3.20) was derived with the assumption that secondary equilibrium effects were absent. This assumption is valid only for nonionizable organic solutes.

With these mathematical relationships presented, we can now discuss the experimental details. The end of a teflon rod was bored out to make a cavity. A volume of 8 μL of a typical organic solvent such as *n*-octane was introduced into the cavity and a cap and rod were fitted to a 1-mL cylindrical vial with a conical bottom to which a magnetic stirrer has been placed. After the solvent is placed on top of the aqueous sample, the sample was stirred for a fixed time period at a fixed temperature, 25°C. This enables the solute to diffuse into the organic solvent. A 1- μL aliquot of this extract is taken and injected into a GC for quantitative analysis. This μLLE yields a β of 0.008. As values of β get smaller and smaller, the second term in the denominator of Eq. (3.20) tends to zero. For a fixed K_D and C_{initial} , a low value for β results in a higher value for C_o and, hence, a higher sensitivity for this μLLE technique.

Once a sample preparation method has been established, the analytical methodology, so important to achieving GLP in TEQA, can be sought. The analytical outcomes as discussed in Chapter 2 can now be introduced for this μLLE technique.

An internal standard mode of calibration was used to conduct quantitative analysis using the minivial technique just described. The analyte studied was 4-methylacetophenone and the internal standard was *n*-dodecane. The slope of the linear calibration was 4.88 L/mmol with a y intercept of zero and a coefficient of determination of 0.998 (17).

15. DOES THE RATE OF MASS TRANSFER BECOME IMPORTANT IN μ LLE?

The kinetics of LLE can also be developed. Kinetics become a more important consideration when aqueous and organic phases cannot be afforded maximum contact, as is the case when a large sep funnel is used. We will consider the principles of kinetics using that recently developed. It is worthwhile to consider kinetics in the context of the μ LLE technique developed by Cantwell and co-workers (18). The general rate equation for LLE can be written in terms of a differential equation that relates the rate of change of the concentration of analyte in the organic phase, C_o , to a difference in concentration between the aqueous phase, $C_{aq}(t)$, and the organic phase $C_o(t)$ to the following:

$$\frac{\partial}{\partial t} C_o = \frac{A}{V_o} \Gamma_o (K_D C_{aq}(t) - C_o(t))$$

where A is the interfacial area and Γ_o is the overall mass transfer coefficient with respect to the organic phase (in units of cm/s). Thus the time dependence of solute concentration in the organic phase can be seen as

$$C_o = C_{o, \text{equil}} (1 - e^{-kt}) \quad (3.21)$$

$C_{o, \text{equil}}$ represents the concentration of solute in the organic phase after equilibrium has been reached. k is the observed rate constant (in units of s^{-1}) and is given by

$$k = \frac{A}{V_o} \Gamma_o [K_D + 1]$$

Combining Eqs. (3.20) and (3.21) leads to an expression that is significant to TEQA:

$$C_{aq, \text{initial}} = C_o(t) \left[\frac{1 + K_D \beta}{K_D (1 - e^{-kt})} \right] \quad (3.22)$$

The term in brackets in Eq. (3.33) is usually held constant and this term is evaluated by extracting a reference aqueous solution where the concentration is known. The concentration must, of course, be in the linear region of the distribution isotherm for both sample and standard.

Cantwell and co-workers have recently extended their μ LLE technique to include a back-extraction using a modification of the minivial discussed earlier. An organic liquid membrane that consists of *n*-octane confined to within a teflon ring “sits” on top of 0.5 or 1 mL of an aqueous sample whose pH is approximately 13 and contains an ionizable analyte. If an amine is dissolved in water and the pH adjusted to 13, the amine would remain unprotonated and therefore, neutral. A large K_D would be expected and the amine should partition favorably into the *n*-octane. A 100- or 200- μ L acidic aqueous phase with a pH of approximately 2 is placed on top of the liquid membrane. The amine is then protonated and “back-extracted” into the acidic aqueous phase (19). A further enhancement utilizes a microliter liquid handling syringe to suspend a drop of acidic aqueous phase within the *n*-octane phase. The syringe that now contains the back-extracted analyte can be directly inserted into the injection loop of a high-performance liquid chromatograph (HPLC).

16. IS THERE ANY OTHER WAY TO PERFORM LLE?

The answer is yes! In TEQA, the sample matrix determines whether LLE involving immiscible solvents is to be used or not. If the sample is a solid, such as a contaminated soil or sediment, the separatory funnel gives way to the Soxhlet extraction apparatus. There have also been attempts to modify the conventional Soxhlet via miniaturization or via instrumentation that pressurizes and heats the extracting solvent. A recent technique promulgated by the EPA is called pressurized fluid extraction. EPA Method 3545 from Update III of SW-846 has been developed to enable priority pollutant semivolatile organics to be isolated and recovered from soils, clays, sediments, sludges, and other solid waste. The Dionex Corporation has developed what they call “accelerated solvent extraction” whereby a much smaller volume of extraction solvent is used. The vial containing the sample and extracting solvent is both heated and pressurized. These extreme temperature and pressure conditions supposedly accelerate the rate at which equilibrium is reached in LLE. The conventional technique for isolating and recovering semivolatile organics from solid matrices is called Soxhlet extraction. Soxhlet extraction as an analytical sample preparation technique has been around for over 100 years! The principle of Soxhlet extraction, abbreviated S-LSE, (because it is a liquid–solid extraction technique), will be discussed in the following section.

17. WHAT IS SOXHLET EXTRACTION ANYWAY?

A solid matrix of environmental interest such as soil that is suspected of containing any of the more than 100 priority pollutant semivolatiles is placed into a cellulosic thimble. Vapors from heating a volatile organic solvent rise and condense above the thimble. This creates a steady-state condition called “reflux.” The refluxed solvent condenses into the thimble and fills until it overflows back into the distilling pot. Reflux is a common technique in organic chemistry and serves to bring the S-LSE process to a fixed temperature. Thus, solutes of interest are able to partition between a fixed weight of contaminated soil and the total extractant volume. Usually, a series of six vessels with six separate heaters are available as a single unit whereby the incoming and outgoing water lines for the reflux condensers are connected in series. A large phase ratio is obtained. S-LSE is usually conducted for one sample over a period of between 12 and 24 h. An overnight continuous S-LSE is quite common. After the extraction time has ended, the glass S-LSE vessel is cooled to room temperature. The extractant in the thimble is combined with the refluxed extractant in the distilling pot. The pot is removed, and due to the large volume of solvent required, the analyst may have over 300 mL of extractant. Common solvents used in S-LSE are, in general, those solvents that are moderate to nonpolar and possess relatively low boiling points. Methylene chloride and petroleum ether are the two most commonly used to conduct S-LSE. Due to the low boiling points, both solvents can be easily removed in the next step after S-LSE.

The extractant from S-LSE must be concentrated. If a low boiling solvent such as methylene chloride is used to conduct S-LSE, it is straightforward to remove this solvent either by use of a Kuderna–Danish (K-D) evaporative concentrator or by use of a rotary evaporator. More contemporary K-D designs provide a means to recover the spent solvent. Earlier designs did not include a means to recover the solvent, and because of this, most solvents such as methylene chloride were evaporated to the atmosphere usually via a fume hood. A few boiling chips are usually added to the receiving tube so as to prevent “bumping” during the vigorous boiling step. Suspending the K-D vessel above a large boiling water bath where steam can be in contact with the glass surface serves to rapidly remove solvent. Solvent is removed until a volume between 1 and 5 mL is reached. An extractant is obtained whose concentration of the analytes of interest has been greatly increased. The concentrated extract is further cleaned up depending on what matrix interferences might be present.

Numerous priority pollutant semivolatiles such as PAHs, various substituted phenols, substituted monoaromatics, and other hazardous chemicals are often extracted along with higher-molecular-weight aliphatic

hydrocarbons. If these hydrocarbons are not removed prior to the determinative step (e.g., separation and quantitation by GC–MS), the MDL can be significantly increased. A peak in the GC for benzo(*a*)pyrene might be obscured because the peak might sit on top of an envelope of hydrocarbon.

Most research papers published during the past 10–15 years that seek to show the value of alternative approaches to S-LSE start by condemning S-LSE as being too labor intensive and consuming too large a volume of extraction solvent. Outside of needing to use approximately 300 mL of volatile solvent as mentioned earlier, this researcher does not share the view that merely filling six thimbles with sample, reassembling the Soxhlet glassware, and turning on the heaters and coolant supply lines is really that time-consuming! After all, once all of this is accomplished, the analyst is free to leave the laboratory and pursue other activities. All the while the sample is continuously refluxed with extractant! Nevertheless, a plethora of research has been done to supplant the classical low-cost Soxhlet, as is discussed next.

18. ARE THERE ALTERNATIVES TO S-LSE?

Yes, and the development of alternatives to conventional S-LSE has occurred during the past 10 years with an emphasis on reducing the extraction solvent volume while maintaining the high efficiency of S-LSE. These newer sample preparation methodologies applied to solid matrices exclusively are variations of S-LSE and include ultrasonic probe liquid–solid extraction (U-LSE), microwave enhanced extraction (MAE) and accelerated solvent extraction (ASE). These techniques assume that the analytical objective is to isolate and recover semivolatile to nonvolatile organic compounds of interest to TEQA from soils, sediments, sludges, and so forth. Beyond these variations to the classical Soxhlet, much progress has been made in performing extractions of solid matrices using supercritical fluids, a technique commonly called supercritical fluid extraction (SFE).

It would be remiss for this author not to include SFE even though SFE is not the forte of this researcher. SFE can be conducted off-line whereby the solid sample is extracted and the extract is then transported to a solvent or adsorbent. In the case of an extract, the analytes of interest being dissolved in the solvent can be directly injected into a GC or, with a change of matrix, injected into an HPLC to complete the analytical steps that lead to TEQA. In the case of a sorbent that contains adsorbed analytes, the sorbent can be eluted with a solvent in much the same way that solid-phase extraction is conducted (refer to the extensive discussion of this technique in subsequent

sections of this chapter). This eluent can then be directly injected into a GC or, with a change of matrix, directly injected into an HPLC in an effort to carry out the determinative step in TEQA. SFE can be interfaced to a chromatograph that uses supercritical fluids as chromatographic mobile phases. The technique is called on-line supercritical extraction–supercritical fluid chromatography, abbreviated SFE–SFC. This technique requires the availability of instrumentation to enable the extracted analytes to be directly injected into the chromatograph where separation of such analytes takes place.

Supercritical fluid extraction as an alternative sample preparation method applied to solid sample matrices of interest to TEQA became quite popular during the late 1980s and early 1990s. However, SFE requires that instrumentation be purchased. It has also been found that a significant matrix dependence exists and this matrix dependence contributes to differences in percent recoveries. The first generation of SFE instruments also suffered from problems with plugging of the flow restrictors that are located after the extraction vessels. SFE will be discussed in more detail later in the chapter. We next discuss the three variations of S-LSE introduced earlier (i.e., U-LSE, MAE, and ASE).

19. WHAT IS ULTRASONIC LIQUID–SOLID EXTRACTION?

Ultrasonic LSE is most applicable to the isolation of semivolatile and non-volatile organic compounds from solid matrices such as soil, sediment, clays, sand, coal tar, and other related solid wastes. U-LSE is also very useful for the disruption of biological material such as serum or tissue. U-LSE can be coupled with solid-phase extraction (SPE) to give a very robust sample preparation method at relatively low cost in comparison to MAE and ASE approaches. The author has utilized U-LSE/SPE to isolate and recover 9,10-dimethyl-1,2-benzanthracene from animal bedding. A 89% recovery was obtained for bedding that was spiked with this polycyclic aromatic hydrocarbon (PAH) of interest to toxicologists (20). An ultrasonic horn and tip are immersed into a mixture of liquid extractant and solid sample and sonicated at some percent of full power for a finite length of time, either continuously or pulsed.

Ultrasonication involves the conversion of a conventional 50 / 60 Hz alternating-current line power to 20 kHz electrical energy and transformation to mechanical vibration. A lead zirconate titanate electrostrictive (piezoelectric) crystal, when subjected to alternating voltage, expands and contracts. This transducer vibrates longitudinally and transmits this motion to the horn tip. The horn tip is immersed in the liquid slurry and cavitation

results. Cavitation is the formation of microscopic vapor bubbles that form and implode, causing powerful shock waves to radiate throughout the sample from the face of the tip. Horns and microtips amplify the longitudinal vibration of the converter and lead to more intense cavitation action and greater disruption. U-LSC dissipates heat and, because of this, a sample should be placed in a ice-water bath. Proper care of the probe is essential. The intense cavitation will, after a prolonged period of time, cause the tip to erode and the power output to decrease without showing up on the power monitor.

Ultrasonic cell disruptors are manufactured by a half-dozen or so companies. In the author's lab, the Model 550 Sonic Dismembrator (Fisher Scientific) has been in use. It becomes important to retune the generator when a new probe is changed. There are also additional tuning procedures to follow for microtips.

EPA Method 3550B (Revision 2, SW-846, December 1996) is a procedure for extracting semivolatile and nonvolatile organic compounds from solids such as soils, sludges, and wastes using U-LSC. The method includes two approaches depending on the expected concentration of contaminants in the sample. In the low-concentration method (< 20 mg each component/kg), a 30-g sample is mixed with anhydrous sodium sulfate to form a free-flowing powder and extracted three times with either 1 : 1 acetone/methylene chloride or 1 : 1 acetone/hexane. The use of a mixed solvent serves to adjust the extractant polarity and thereby enabling efficient extraction of some of the more polar analytes as well. In the high-concentration method (> 20 mg each component/kg), a 2-g sample is mixed with anhydrous sodium sulfate, again, to form a free-flowing powder. The extracts are concentrated by evaporating off the solvent using Kuderna–Danish evaporative concentrators. A solvent-recovery system is recommended whereby the vaporized solvent can be recycled to prevent escape to the atmosphere. Surrogates and matrix spikes are added to the free-flowing powder once sodium sulfate has been added. Solvent evaporation can be combined with a solvent exchange depending on which determinative technique is to be used. All solvents used should be pesticide residue grade or equivalent. If further concentration of the extract is needed, either a micro Snyder column technique or a nitrogen blowdown technique is used.

Percent recoveries for 21 representative semivolatile priority pollutant organic compounds are listed in the method taken from the categories of base (B), neutral (N), and acid (A), the so-called BNAs from the over 100 compounds that are routinely monitored for in EPA-contract-type work.

The realization that the physicochemical conditions of the extract could be altered led to the development of accelerated solvent extraction (ASE) or pressurized fluid extraction whereby the same solvents used to

conduct S-LSE are used. The elevation of the extract temperature and pressure serve to “accelerate” the mass transfer of analyte from solid matrix to extract and hence reduce the time it takes to achieve a high percent recovery. The chemical nature of the extractant can also be changed, such as using carbon dioxide as a supercritical fluid. This is accomplished by elevating its temperature to slightly above its critical temperature point while increasing its pressure to slightly above its critical pressure. This sample prep technique called supercritical fluid extraction (SFE) is another alternative to S-LSE as applied to samples that are solids. We are going to digress a bit to discuss the underlying principles of ASE and SFE.

20. CAN PHASE DIAGRAMS HELP TO EXPLAIN THESE ALTERNATIVE SAMPLE PREP TECHNIQUES?

The answer is yes and we will digress a bit at this point to introduce these concepts as we did earlier in the chapter. The temperature and pressure conditions that govern physico-chemical behavior of liquids are defined in terms of thermodynamics. The Gibbs Phase Rule is a direct outcome of the physical chemistry of changes in the state of matter. The phase rule helps to interpret the physico-chemical behavior of solids, liquids, and gases within the framework of the kinetic-molecular theory of phase equilibria.

If there are c distinct chemical species or components, the composition of any one phase is specified by $c - 1$ mole fractions (21). The composition of the remaining component is fixed. For p phases, the number of composition variables which must be independently assigned is

$$\# \text{ Composition variables} = p(c - 1)$$

In addition to the required composition variables, the two remaining parameters that change the thermodynamic state if varied are temperature and pressure. Hence,

$$\text{Total \# of variables} = p(c - 1) + 2$$

Not all of these variables are necessary to define a system; not all of these variables are independent. The fact that the chemical potential for a given component must be the same in every coexisting phase places “restrictions” on the number of independent variables necessary; hence, for a given component present in three phases (1, 2, and 3) stated mathematically,

$$\left(\frac{\partial G}{\partial n_1}\right)_{T,P} = \left(\frac{\partial G}{\partial n_2}\right)_{T,P} = \left(\frac{\partial G}{\partial n_3}\right)_{T,P}$$

Two equations are needed to satisfy the above condition for three phases or $p - 1$ equations or restrictions per component. For c components, we have

$$\# \text{ of restrictions} = c(p - 1)$$

The number of parameters that can be independently varied, f , is found from the difference between the total number of variables and the total number of restrictions. Stated mathematically,

$$\text{Total \# of independent variables} = p(c - 1) + 2 - [c(p - 1)]$$

The smallest number of independent variables that must be specified in order to describe completely the state of a system is known as the number of degrees of freedom, f . For a fixed mass of a gas, $f = 2$, because one can vary any two variables (e.g., pressure and temperature); the third variable is fixed by the equation of state (e.g. volume). Hence, only two properties of a fixed mass of gas are independently variable. Stated mathematically, for a system at equilibrium, the number of degrees of freedom, f , equals the difference between the number of chemical components, c , and the number of phases, p (22):

$$f = c - p + 2$$

This is, in essence, the celebrated Gibbs Phase Rule. A generalized version of a phase diagram for a one component system, $c = 1$, whereby the pressure exerted by the substance is plotted against the temperature of the substance is shown in Figure 3.7. This means that different regions of the phase diagram yield different values for f . Those regions that P and T are such that only one phase exists have $f = 2$ and this means that both P and T can be varied independently. On one of the phase change lines, $f = 1$ and this means that if T were changed, P could not be changed independently of T if the two phases are to remain in equilibrium; rather, P must change in such a way as to keep the point on the line. At the triple point, $f = 0$, and there is a unique value for P and T . At this triple point, P and T can have only one value and only at this point are solid, liquid, and gas allowed to exist in equilibrium. The phase diagram for most chemically pure substances exhibit a slight tilt to the right for their solid to liquid change of phase, the so-called fusion line. One notable exception is that of water whose fusion line tilts slightly to the left. This enables a skater to apply a large enough pressure

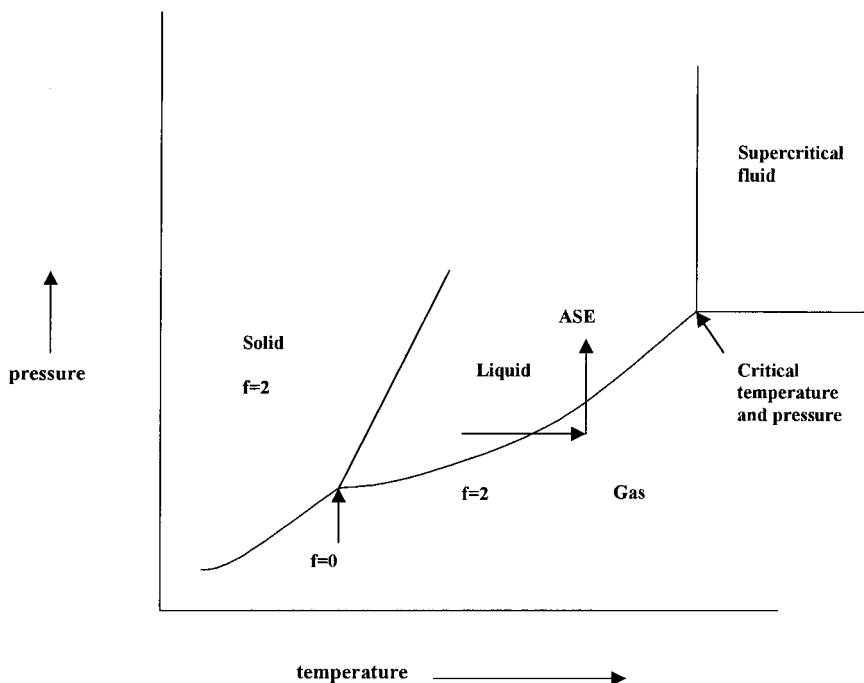


FIGURE 3.7 Generalized phase diagram for any substance.

onto the surface of ice to enable melting to occur and thus provide sufficient lubrication. Note the horizontal line that crosses the liquid–gas transition. This line indicates that the temperature of the extractant is being increased. To prevent vaporization from occurring, the vertical line shows that the pressure must be increased to again cross over the liquid–gas transition and keep the extractant as a liquid. This is exactly how the ASE operates and the phase diagram in Figure 3.7 is so noted. The region beyond the critical temperature and pressure, labeled as the supercritical fluid region, is where SFE is conducted. Phase diagrams for carbon dioxide, the most common substance for performing SFE, are well established. Let us examine ASE a little further.

21. HOW DOES ASE WORK?

A solid sample such as soil, whose priority pollutant content is of interest, is placed in a vessel that is capable of withstanding high pressures and tem-

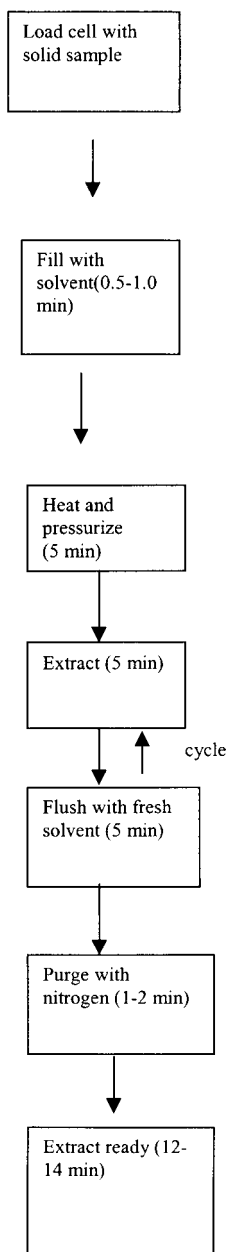
peratures. The vessel is filled with the extractant and extracted under elevated temperature (50–200°C) and pressure (500–3000 psi) for relatively short periods of time (5–10 min). The technique was first published by Richter et al., who also discusses the physico-chemical considerations that went into developing ASE (23). The solubility of organic compounds in organic solvents increases with an increase in solvent temperature. Faster rates of diffusion also occur with an increase in solvent temperature. If fresh extracting solvent is introduced, the concentration gradient is increased and this serves to increase the mass transfer rate or flux according to Fick's first law of diffusion. Thermal energy can overcome cohesive (solute–solute) and adhesive (solute–matrix) interactions by decreasing the energy of activation required for the desorption process. Higher extractant temperatures also increase the viscosity of liquid solvents and thus allow better penetration of matrix particles and enhancing extraction. Increased pressure enables liquids to exist at the elevated temperatures. Increased pressure forces the solvent into areas of the matrix that would not normally be contacted by solvents using atmospheric conditions.

Instrumentation to set up and perform ASE on solid matrices of interest to TEQA has been commercially available for several years from Dionex Corporation. The instrumentation to perform ASE is quite expensive and for this reasons, labs should have a large sample workload so that return on investment can be realized in as short a time frame as possible.

The ASE instrument works as follows. Solvent is pumped into the extraction cell after the cell is filled with the solid sample. The cell is heated and pressurized to enable the extractant to remain as a liquid well above its boiling point. A finite amount of time is taken for LSE to occur. While hot, the cell is flushed one or more times with solvent and the extractant is transferred to a collection vial. The remaining solvent is purged out of the cell with compressed nitrogen. These steps have been recently introduced and are outlined in the flow chart in Scheme 3.1 (24):

22. WHAT SAMPLES HAS ASE BEEN APPLIED TO?

Two studies from the original paper by Richter et al. will now be discussed (23). The first study involved isolating and recovering total petroleum hydrocarbons (TPHs) from soil using ASE. Four different extraction temperatures were used. TPHs were determined via infrared absorption. The quantitative analysis of oil-laden soils has been discussed in Chapter 2 and the determinative technique for this will be discussed in Chapter 4. The results are shown in Table 3.1 and demonstrate that extractant temperature can significantly influence TPH recoveries. The precision over five replicate



Scheme 3.1 Steps in operation of the ASE.

TABLE 3.1 Effect of Temperature on the Recovery of TPHs from Soil Using ASE

Extractant temp. (°C)	Amount found (mg) (<i>n</i> = 5)	RSD (%)
27	974	6.0
50	1118	5.0
75	1190	2.0
100	1232	1.0

Note: ASE conditions:

- Preheat method
- Perchloroethylene as extractant
- 2500 psi, 5 m in static extraction after equilibration
- 3g soil
- 3.5 mL cell volume
- 4.5–5.0 mL solvent used
- 1200 mg TPH/kg soil

ASEs is seen to decrease as the extractant temperature is increased. The second study involved isolating and recovering the selected polycyclic aromatic hydrocarbons (PAHs) from a certified reference sample of urban dust.

Table 3.2 shows the results and lists the ASE conditions for this study. Nearly 100% recoveries are reported together with good precision (% RSDs < 7% for six replicate ASEs) for these 10 priority pollutant PAHs.

Two validation studies conducted in 1994 compared ASE with S-LSE, automated Soxhlet, and wrist-shake or manual LLE for the determination of target analytes from solid wastes of interest to TEQA. These results are summarized in Table 3.3. ASE proved equivalent to the conventional procedures. The data generated in these two studies resulted in the generation of EPA Method 3545 that received final promulgation in June 1997 as part of SW-846 Method Update III (25).

We now turn our attention to sample preparation techniques to quantitatively determine volatile organic compounds (VOCs) in environmental samples, and after this, we return to a discussion of the more recently developed sample prep techniques for SVOCs. Because we have been discussing LLE and LSE techniques, it is appropriate for us to ask at this point the following question.

TABLE 3.2 Recovery of PAHs from Urban Dust (SRM 1649)

PAH	Mean % recovery (<i>n</i> = 6)	RSD (%)
Phenanthrene	113	2.0
Fluoranthene	88.5	5.2
Pyrene	91.0	5.9
Ben[<i>a</i>]anthracene	97.2	5.7
Chrysene	101	4.2
Benzo[<i>b</i>]fluoranthene	115	6.2
Benzo[<i>k</i>]fluoranthene	112	6.7
Benzo[<i>a</i>]pyrene	125	6.2
Benzo[<i>ghi</i>]perylene	108	5.8
Indeno[1, 2, 3- <i>cd</i>]pyrene	108	6.7

Note: ASE conditions:

- Prefill method
- 100°C
- Methylene chloride/acetone 1 : 1 (v/v)
- 5 min equilibration, 5 min static
- 18 mL final volume
- 2000 psi

TABLE 3.3 Summary of Laboratory Validation Studies Used for Validation of ASE for EPA Method 3545

Compound class	Matrix	Comparison technique	Relative recovery (%)
Organochlorine pesticides	Clay, loam, sand	Automated Soxhlet (Method 3541)	97.3
Semivolatile compounds	Clay, loam, sand	Automated Soxhlet (Method 3541)	99.2
Organophosphorous pesticides	Clay, loam, sand	Soxhlet (Method 3540)	98.6
Chlorinated herbicides	Clay, loam, sand	Manual LLE (Method 8150)	113
PCBs	Sewage sludge, river sediment, oyster tissue, soil	Various certified reference materials	98.2
PAHs	Urban dust, marine sediment, soil	Various certified reference materials	105

23. CAN VOLATILE ORGANICS BE ISOLATED FROM CONTAMINATED WATER USING LLE?

The answer is yes. Since the original discovery of THMs in the city of New Orleans, drinking water was determined by conducting LLE using pentane as the extracting solvent (26). If the sample contaminants are well known in a particular environmental sample, it is possible to use LLE as the principal sample prep technique. LLE is, however, unselective, and semi-volatile and nonvolatile organics are equally likely to be extracted in samples that contain unknown contaminants. Consistent with the EPA protocols introduced in Chapter 1, methods have been developed to remove VOCs either by purging or by sampling the headspace in a sealed vial that contains the contaminated groundwater sample. The former technique will be discussed after we develop the principles behind the latter technique, commonly called static headspace sampling for the determination of trace volatile organics (VOCs), in drinking water, groundwater, surface water, wastewater or soil.

24. ON WHAT BASIS CAN VOCs BE QUANTITATED USING STATIC HEADSPACE TECHNIQUES?

Liquid-liquid extraction has a counterpart for the determination of VOCs in the technique known as static or equilibrium headspace sampling. The technique is most often combined with the determinative step and is often referred to as static headspace gas chromatography, abbreviated HS-GC. The principles that underlie this technique will be outlined in this section. The decision to measure VOCs in the environment by either HS or purge and trap (P&T or dynamic headspace) represents one of the ongoing controversies in the field of TEQA. This author has worked in two different environmental laboratories in which one used P&T as the predominant technique to determine VOCs in the environment and the other used HS-GC.

Let us start by introducing the thermodynamic viewpoint for the distribution of a substance originally dissolved in the sample. The substance exists as a gas at room temperature in a closed vessel between an aqueous phase and a gaseous phase. The gaseous phase in HS usually consists of air saturated with water vapor. Dissolved gases and volatile organic compounds are the two most common chemical substances that are likely to be found in the headspace. Dissolved gases equilibrate into the HS because they follow Henry's Law. Volatile organic compounds, many of which are classified as priority pollutants by the EPA, behave similarly to that of fixed

gases and each different VOC has its own Henry's Law constant, K_H . We will consider sulfur dioxide as being illustrative of a dissolved gas and proceed to develop the basis for this equilibrium. We will next use trichloroethylene as illustrative of a dissolved VOC and develop the more applied equations that must be considered in order to relate HS techniques to TEQA.

25. WHAT HAPPENS WHEN GASEOUS SO_2 IS DISSOLVED IN WATER, THEN PLACED IN A VESSEL AND SEALED?

Consider a water sample that contains dissolved SO_2 . The sample is placed in a cylindrical glass vessel and then sealed. Glass vials with a crimped top seal and a volume of either 22 mL or 40 mL are commonplace. If the pH of the aqueous phase could be measured, the value would indicate that the effect of the dissolved gas acidified the water. It is well known that one molecule of SO_2 combines with one molecule of water to produce sulfurous acid according to



Sulfurous acid, in turn, is known to ionize according to



Both equations describe what we have been calling secondary equilibrium effects to the primary distribution equilibria, which, in this case, is the distribution of neutral SO_2 molecules between the aqueous phase and the HS described as follows:



The extent to which SO_2 partitions into the HS is determined by the Henry Law constant, K_H .

In a manner similar to that developed for LLE [i.e., refer to Eqs. (3.1) and (3.2)], it becomes apparent that in a closed system between two phases, the chemical potentials for SO_2 in each phase becomes equal once dynamic equilibrium is reached. If this is so, then similar to that stated earlier, we can mathematically state

$$\mu_{\text{HS}, \text{SO}_2} = \mu_{\text{aq}, \text{SO}_2}$$

The chemical potential for SO_2 in either phase is related to a standard state chemical potential, μ^0 for SO_2 in both the headspace (HS) and in the aqueous phase and a term related to either the pressure exerted by the gas in the HS or the activity in the dissolved state according to

$$\mu_{\text{HS},\text{SO}_2}^0 + RT \ln p^{\text{SO}_2} = \mu_{\text{aq},\text{SO}_2}^0 + RT \ln a_{\text{aq}}^{\text{SO}_2}$$

We seek to express the ratio of activity to partial pressure so as to be consistent with a subsequent definition of the partition coefficient. Upon rearranging, we obtain the following relationship:

$$RT \ln \left(\frac{a_{\text{aq},\text{SO}_2}}{p_{\text{HS},\text{SO}_2}} \right) = -(\mu_{\text{aq},\text{SO}_2}^0 - \mu_{\text{HS},\text{SO}_2}^0)$$

Upon further rearranging and proceeding to define the Henry Law constant, we obtain

$$\frac{a_{\text{aq},\text{SO}_2}}{p_{\text{HS},\text{SO}_2}} = e^{-\Delta\mu^0/RT} \equiv K_H^{\text{SO}_2} \quad (3.23)$$

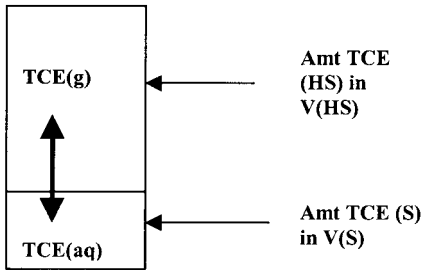
Secondary equilibrium effects are handled by defining the degree of ionization by α as done previously, refer to Eqs (3.17) and (3.18), and expressing the activity of neutral SO_2 in terms of α and the total activity, a_T , according to

$$a_{\text{SO}_2} = a_T(1 - \alpha)$$

For example, if 0.7643 mol SO_2 is dissolved in 1 kg water in a closed system, a 0.7643 *M* solution results, and with $\alpha = 0.1535$, the vapor pressure exerted by SO_2 is such that K_H is 0.813. When 1.0273 g SO_2 is dissolved in the same weight of water with α this time being 0.1204, the vapor pressure exerted is such that K_H is again 0.813 (27).

26. ON WHAT BASIS CAN WE QUANTITATE TCE IN GROUNDWATER BY HS-GC?

Consider how the priority pollutant VOC trichloroethylene or trichloroethene, abbreviated TCE, is distributed between a sample of groundwater and the headspace in a sealed HS vial as shown schematically by:



Let us assume that the temperature is fixed such as would be found in a thermostatted automated HS analyzer. A consideration of mass balance requires that the original amount of TCE must be found either in the headspace or the aqueous sample. Mathematically stated,

$$\text{amt}_o^{\text{TCE}} = \text{amt}_S^{\text{TCE}} + \text{amt}_{\text{HS}}^{\text{TCE}} \quad (3.24)$$

Using principles encompassed by Eq. (3.23), we can proceed to define the partition coefficient as

$$K^{\text{TCE}} \equiv \frac{C_S}{C_{\text{HS}}} \quad (3.25)$$

It is helpful at this point to define the abbreviations used to develop the equations that lead to Eq. 3.26 derived below. Consider the following definitions:

- o refers to the original concentration of TCE in the groundwater sample.
- S refers to the sample.
- HS refers to the headspace.
- amt refers to an amount of TCE.
- V_S refers to the volume of sample placed in the HS vial.
- V_{HS} refers to the volume of headspace in the HS vial after V_S mL have been placed in the HS vial.
- C refers to a concentration.
- β refers to the phase ratio for a HS vial and equals V_{HS}/V_S .

We can proceed from both the mass balance principle and from the definition of K^{TCE} . Let us divide Eq. (3.24) by V_S :

$$\begin{aligned}
\frac{\text{amt}_o}{V_S} &= \frac{\text{amt}_S}{V_S} + \frac{\text{amt}_{\text{HS}}}{V_S} \\
C_o &= C_S + \frac{V_{\text{HS}} C_{\text{HS}}}{V_S} \\
&= K_H^{\text{TCE}} C_{\text{HS}} + \frac{V_{\text{HS}}}{V_S} C_{\text{HS}} \\
&= C_{\text{HS}} \left[K_H^{\text{TCE}} + \frac{V_{\text{HS}}}{V_S} \right] \\
&= C_{\text{HS}} \left[K_H^{\text{TCE}} + \beta \right]
\end{aligned} \tag{3.26}$$

Solving Eq. (3.26) for C_{HS} gives a useful relationship:

$$C_{\text{HS}}^{\text{TCE}} = C_o \left[\frac{1}{K_H^{\text{TCE}} + \beta} \right] \tag{3.27}$$

Equation (3.27) suggests that for a given concentration of TCE in the original sample of groundwater, the concentration of TCE expected to be found in the HS, $C_{\text{HS}}^{\text{TCE}}$, depends not only on C_o but also on two factors: K^{TCE} , which is due to the physico-chemical nature of this particular VOC, and β , which is due to the volumes occupied by HS and sample. β might be thought of in terms of the physical characteristics of the HS vial. In practice, however, K^{TCE} may exhibit some dependence on temperature and sample ionic strength, whereas the range of values that β can take on is limited. As we shall see, both factors play off of one another in the consideration of analyte sensitivity in HS techniques.

Equation (3.27) is the basis for TEQA, because for a given VOC and fixed sample volume in a headspace vial (i.e., $K_{\text{HS}}^{\text{VOC}}$ and β), $C_{\text{HS}}^{\text{VOC}}$ is directly proportional to the original concentration of VOC in the aqueous sample, C_o . The volume of headspace sampled and injected into a GC is usually held fixed so that the area under the curve of a chromatographically resolved GC peak, A^{VOC} is directly proportional to $C_{\text{HS}}^{\text{VOC}}$. As discussed in Chapter 2, Eqs. (2.1) and (2.2), with respect to the external mode of instrument calibration, the *sensitivity* of the HS-GC technique is related to the magnitude of the response factor, R_F^{HS} according to

$$R_F^{\text{HS}} = \frac{A_{\text{HS}}^{\text{VOC}}}{C_{\text{HS}}^{\text{VOC}}}$$

A fixed aliquot of the HS whose concentration of TCE, $C_{\text{HS}}^{\text{TCE}}$, is injected into a GC with a halogen-specific detector such as an electrolytic conductivity detector (EICD) (refer to EPA Methods 601 and 602 even though these methods employ P&T techniques). Alternatively, this aliquot can be injected into a gas chromatograph–mass spectrometer (GC–MS). The mass spectrometer might be operated in the selective ion monitoring (SIM) mode. The most abundant fragment ions of TCE would then be detected only (refer to EPA Method 624 even though this method employs P&T techniques). Determinative techniques such as GC–EICD and GC–MS are introduced in Chapter 4. Nevertheless, it should be apparent to the reader at this point that the partition coefficient plays an important role in achieving a high sensitivity in HS techniques. We digress from here to discuss this a bit more.

27. ON WHAT BASIS DOES K^{TCE} DEPEND?

Let us approach an equivalent to Eq. 3.24 from the perspective of applying the three great laws of phase equilibrium found in most physical chemistry texts: Dalton's Law of partial pressures, Raoult's Law of ideal solutions, and Henry's Law for dissolved gases (28). Applying Dalton's law enables one to state that the concentration of analyte in the HS is proportional to its partial pressure. The partial pressure exerted by TCE in the HS is independent of all other gases in the HS mixture and is related to the total pressure in the HS as follows:

$$p_i^{\text{HS}} = p_T X_i^{\text{HS}}$$

This partial pressure exerted by component i is, in turn, related to the vapor pressure exerted as if i were pure, p_i^o and the mole fraction of component i dissolved in the sample, X_i^S according to Raoult's Law. An activity coefficient γ_i is included to account for nonideality so that

$$p_i^{\text{HS}} = p_i^o \gamma_i X_i^S$$

Also, the partial pressure exerted by component i can be related to the mole fraction of i dissolved in the sample, S , according to Henry's Law as follows:

$$p_i^{\text{HS}} = K_H X_i^S$$

The partial pressure of component i in the HS can be eliminated so that

$$p_T X_i^{\text{HS}} = p_i^o \gamma_i X_i^{\text{S}}$$

Rearranging this equation to a ratio of mole fractions yields

$$\frac{X_i^{\text{S}}}{X_i^{\text{HS}}} = \frac{p_T}{p_i^o \gamma_i} \approx \frac{C_i^{\text{S}}}{C_i^{\text{HS}}} = K$$

This equation yields an alternative relationship for the partition constant in HS in which K is found to be inversely proportional to a product of the partial pressure of component i if it were pure and its activity coefficient γ_i according to

$$K \propto \frac{1}{p_i^o \gamma_i} \quad (3.28)$$

Because Eq. (3.25) relates K to a ratio of concentrations, combining Eqs. (3.25) and (3.28) leads to the following:

$$\frac{C_i^{\text{S}}}{C_i^{\text{HS}}} \propto \frac{1}{p_i^o \gamma_i} \quad (3.29)$$

P_{TCE}^o refers to the intrinsic volatility exhibited by a chemically pure substance TCE. TCE is a liquid at room temperature, and if one opens a bottle that contains the pure liquid, one is immediately “struck” by virtue of the sense of smell with the concept of the vapor pressure of TCE! It is likely that P_{TCE}^o is greater than P_{PCE}^o , where PCE refers to perchloroethylene (tetrachloroethene). PCE is also on the EPA’s priority pollutant list. One would expect to find that $C_{\text{TCE}}^{\text{HS}}$ is greater than $C_{\text{PCE}}^{\text{HS}}$, assuming all other factors equal. This is largely due to the inverse relationship between K and p^o , as shown in Eq. (3.28). This equation also suggests that if a matrix effect exists, K is also influenced due to differences in the activity coefficient, γ . Changes in γ might be due to changes in the ionic strength due to the sample matrix and, in turn, influences K , as shown in Eq. (3.28).

28. MUST WE ALWAYS HEAT A SAMPLE IN HS-GC TO CONDUCT TEQA?

Equations (3.27)–(3.29) lay the foundation for a theoretical understanding of what factors are involved in obtaining a sufficiently large value for the concentration of any VOC in the HS (i.e. $C_{\text{HS}}^{\text{VOC}}$). In this section, we look at the effect of increasing temperature of a headspace vial that contains prior-

ity pollutant VOC dissolved in water. An example of this might be a sample of groundwater that has been contaminated with priority pollutant VOCs. The static equilibrium HS technique just described would be used to satisfy the criteria for TEQA.

The Clausius–Clapeyron equation, one of the most famous in physical chemistry, is most applicable for this discussion. The equation states that the partial differential with respect to absolute temperature of the logarithm of a pure liquid’s vapor pressure is inversely related to the liquid’s absolute temperature. We again consider the liquid TCE and state the Clausius–Clapeyron equation mathematically (29):

$$\frac{\partial}{\partial T}(\ln p^o) = \frac{\Delta H_v}{RT^2}$$

Upon rearranging and expressing this relationship with respect to the VOC that we are considering, TCE, we obtain

$$\ln p_{\text{TCE}}^o = \frac{\Delta H_v^{\text{TCE}}}{R} \int \frac{dT}{T^2}$$

The indefinite integral is then evaluated as

$$-\frac{\Delta H_v^{\text{TCE}}}{RT} + C$$

The vapor pressure exerted by pure TCE can then be expressed as

$$p_{\text{TCE}}^o = e^{\left[-\left(\frac{\Delta H_v^{\text{TCE}}}{RT} \right) + C \right]} \quad (3.30)$$

where ΔH_v^{TCE} is the molar heat of vaporization for pure TCE and R is the ideal-gas constant. Equation (3.30) suggests that a pure liquid’s vapor pressure increases exponentially as the temperature of the liquid is increased, until its vapor pressure reaches that exerted by the atmosphere. A plot of Eq. (3.30) is shown in Figure 3.8 for three different liquids. For example, if three ClVOCs are plotted such as that for chloroform, TCE, and PCE, their respective vapor pressure/temperature curves would resemble the three shown in Figure 3.8. Because the partition coefficient and the partial pres-

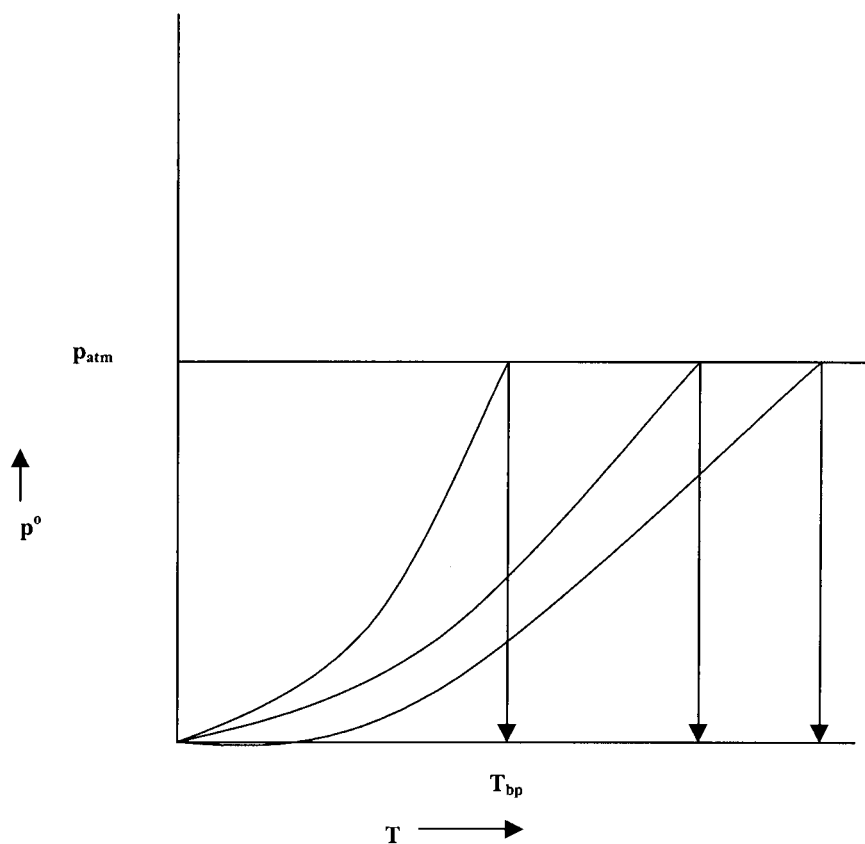


FIGURE 3.8 Plot of the vapor pressure of a liquid versus the liquid's temperature.

sure of a pure liquid such as TCE are inversely related according to Eq. (3.28), we can relate K and T in the following manner:

$$\ln K_{\text{HS}}^{\text{TCE}} = \frac{A}{T} - B \quad (3.31)$$

where A and B are constants related to TCE. Thus, an increase in T serves to decrease $K_{\text{HS}}^{\text{TCE}}$ and according to Eq. (3.29) partitions a greater percent of more TCE molecules into the HS versus the condensed phase. In other words, the ratio $C_{\text{S}}^{\text{TCE}}/C_{\text{HS}}^{\text{TCE}}$ is seen to decrease.

29. IS THERE AN EXAMPLE THAT ILLUSTRATES THE CONCEPT IN EQ. (3.31)?

One application of these principles is evident in the hypothetical preparation of a mixture of C_5 through C_{20} alkanes; each alkane is then placed in a HS vial in equal amounts. Prior to sealing the vial, a 10- μ L aliquot of the liquid is taken and injected into a gas chromatograph with a carbon-selective detector. A chromatogram is generated that shows a separate peak for each of the 16 alkanes. The HS vial is then sealed with a crimped top cap and septum. The vial is placed inside a cylindrical heater block whose temperature has been set previously to 80°C. A 1.0cc gas-tight syringe is used to withdraw an aliquot of the headspace and directly injected into the same GC. A chromatogram is generated that shows a distribution of the 16 peaks that is different from the first. The lower-carbon-number or lower-molecular-weight alkanes seem to have been enriched in the HS as compared to the direct liquid injection. These results are readily explained by considering Eq. (3.26) and the subsequent reexpression of Eq. (3.30) in terms of the HS partition coefficient [i.e. Eq. (3.31)].

30. WHAT HAPPENS WHEN K_{HS}^{VOC} VALUES VARY SIGNIFICANTLY?

Kolb and Ettre recently discussed their work on determining values for K_{HS}^{VOC} and report some interesting findings. They observed that over a range of some 15 VOCs that vary in polarity from dioxane, to lower-molecular-weight alcohols through to ketones and then to acetate-type esters, to monoaromatics and to polychlorinated ethenes, and, finally to C_6 alkanes that K_{HS}^{VOC} values vary by over four orders of magnitude (30)! Those VOCs that exhibit low K_{HS}^{VOC} values (i.e. favor a high concentration in the HS) (C_{HS}^{VOC} is high at equilibrium) are not influenced by an increase in temperature. Those VOCs that exhibit high K_{HS}^{VOC} (i.e., favor a low concentration in the HS) (C_{HS}^{VOC} is low at equilibrium) are strongly influenced by an increase in temperature. Kolb and Ettre chose five solutes as representative of the large number of VOCs that have a wide range of partition coefficients. These are listed below along with their partition coefficients:

Solute	K_{HS}^{VOC} at 40°C
Ethanol	1355
Methyl ethyl ketone	139.5 at 45°C
Toluene	2.82
Tetrachloroethylene	1.48
<i>n</i> -Hexane	0.14

As the temperature is increased while β is held fixed at 3.46 from 40°C to 80°C, the partition coefficient for ethanol is observed to increase to the greatest degree, whereas that for methyl ethyl ketone is also increased but to a lesser degree. Partition coefficients for toluene, tetrachloroethylene, and *n*-hexane do not increase to any extent as the temperature is increased from 40°C to 80°C.

This phenomenon is explained by these authors in the following manner. VOCs whose partition coefficients are already low, are enriched in the HS at the lower temperature, 40°C, and the percent of remaining dissolved VOCs in the condensed phase that partition into the HS as the temperature is raised to 80°C is not appreciable. Polar solutes, on the other hand, are predominantly dissolved in the condensed phase at 40°C and the percent that partition into the HS as the temperature is raised to 80°C is much more appreciable.

Let us return to Eq. (3.27) and briefly consider the second factor, the phase ratio β , in the headspace sampling of groundwater to determine trace concentrations of VOCs in the environment. Kolb and Ettre have studied the influence of β on HS sensitivity. For a fixed temperature and fixed original concentration of VOC in the aqueous phase, C_o , the influence of changing β from, for example, 4.00 (only 20% of the total volume of the HS vial contains the sample) to $\beta = 0.250$ (80% of the total volume of the HS vial contains the sample) depends on the magnitude of K_{HS}^{VOC} . For nonpolar aliphatic hydrocarbons like *n*-hexane or for chlorinated C_1 or C_2 aliphatics such as TCE, a change in β from 4.00 to 0.250 increases the HS sensitivity by almost a factor of 10! For monoaromatics, the same change in β gives an increase in HS sensitivity of about 4. For acetate-type esters, ketones, lower-molecular-weight alcohols and ethers, a change in β from 4.00 to 0.250 gives an insignificant increase in HS sensitivity (30).

The influence of the sample matrix activity coefficient, γ , represents the third factor that serves to influence HS sensitivity. The theoretical basis for this is encompassed in Eq. (3.28) or (3.26). The influence of increasing γ by adding salt to an aqueous sample, a well-known technique called *salting out*, has been shown to have a negligible effect on nonpolar VOCs such as TCE dissolved in water. Polar solutes, however, are more strongly influenced by changes in the sample matrix activity coefficient. It has also been observed that a high concentration of salt must be dissolved in the aqueous sample matrix to have any effect at all! The addition of a high amount of salt increases the volume of the liquid sample and thus serves to decrease β . There are a number of drawbacks to adding salt in static HS, including increases in sample viscosity and the addition of volatile impurities.

Few comprehensive studies have been published on the effect of the sample matrix on the partitioning of VOCs at trace concentration levels.

Friant and Suffet chose four model compounds that are polar and representative of the intramolecular forces of dispersion, dipole orientation, proton-donor capability, and proton-acceptor capability (31). These were methyl ethyl ketone, nitroethane, n-butanol and p-dioxane. The pH of the aqueous sample matrix had no effect on all except for nitroethane. An optimum salt concentration was 3.35M in sodium sulfate and an optimum HS sampling temperature of 50°C. For example, the partition coefficient of methyl ethyl ketone increased from 3.90 to 260 when the temperature of the aqueous sample that contained the dissolved ketone was increased from 30°C to 50°C and at the same time, the concentration of salt increased from zero to 3.35M. Otson et al. compared static HS, P&T, and LLE techniques for the determination of THMs in drinking water and found that static HS showed the poorest precision and sensitivity. However, a manual HS sampling technique was used back in this era and might possibly have contributed to this loss of precision. LLE was found to be quite comparable to P&T in this study (32).

31. WHY IS STATIC HEADSPACE SAMPLING NOT MORE ACCEPTABLE TO THE EPA?

This might be close to the \$64,000 question! The answer lies somewhere between “MDLs are not low enough in comparison to those obtained using P&T” and HS techniques “were not developed in EPA labs while purge and trap techniques were.” Static HS whose principles have already been introduced in this chapter requires that analytes be classified as being volatile (i.e., having boiling points < 125°C). As also discussed, the fundamental principles behind static HS techniques are only recently becoming understood. Most static HS techniques are performed in automated analyzers. These analyzers are directly interfaced to the injection port of a gas chromatograph via a transfer line. This “on-line” instrumental configuration does not allow for the GC’s IDL to be experimentally measured independently, as is done for “off-line” LLE in the determination of semivolatile analytes. Thus, the GC detector that is available is crucial to the notion of HS sensitivity. Because of the low detector response factor, a given VOC can be highly favored to partition into the headspace, yet have a MDL that is less than desirable. In the author’s laboratory, the technique of manual HS sampling is popular among environmental microbiologists who need analytical results almost immediately after they sample the headspace of their bioreactors. These researchers cannot wait for the long equilibration times of commercial automated HS analyzers. P&T techniques would likewise be out of the question as a means to measure trace VOCs, due to the long purging, trapping, and thermal desorbing times involved.

The difficulty that the EPA has had with the static headspace technique might be seen in the comment from Method 3810 (SW-846 3rd edition). This method was eliminated in the recently published final update (III) and replaced by Method 5021. The authors who wrote Method 3810 state (33):

Detection limits for this method may vary widely among samples because of the large variability and complicated matrices of waste samples. . . . The sensitivity of this method will depend on the equilibria of the various compounds between the vapor and dissolved phases. . . . Due to the variability of this method, this procedure is recommended for use only as a screening procedure for other, more accurate determinative methods . . .

Method 3810 recommends that a sample be heated to 90°C and 2 mL of headspace gas taken with a gas-tight syringe. The written procedure for this method was published in SW-846 and provided no data on method performance at that time. The publication of a method from a regulatory agency without even a minimum of quality assurance and quality control is inexcusable! This method serves as one example of neglected GLP. The importance of GLP was introduced in Chapter 1 and this neglect attests to the fact that little to no interest in static HS techniques existed back in 1986.

32. IS THERE A RECENTLY UPDATED EPA METHOD FOR VOC USING STATIC HS TECHNIQUES?

Yes there is and it is Method 5021 from the recently updated SW-846 series of methods published by the Office of Solid Waste at EPA. The method uses the static HS technique to determine VOCs from soil or other solid matrix. This section will focus on some of the details of this method because it includes many of the quality control (QC) features that were absent in the method just discussed. This method also introduces some experimental considerations with respect to trace VOC analyses of soil samples (34). The method is applicable to a wide range of organic compounds that have sufficiently high volatility to be effectively removed from soil samples using static HS techniques. The method is used in combination with a determinative technique that is described in the 8000 series. The method cautions the user to the fact that solid samples whose organic matter content exceeds 1% or for compounds with high octanol/water partition coefficients may yield a lower result for the determination of VOCs by static HS in comparison to dynamic headspace (P&T). It is

recommended to add surrogates to each and every sample to evaluate the so-called “matrix effect.”

33. HOW SHOULD I PROCEED TO QUANTITATE VOCs?

The EPA’s approach to sample preparation to determine trace concentration levels of various VOCs is discussed in this section. We have already introduced static HS techniques from a theoretical point of view. Purge and trap (P&T) techniques, in contrast to static HS, involve passing an inert gas through an aqueous sample such as drinking water or groundwater. The purge gas is directed to a “trap”, which is a term commonly used to describe a packed column that contains an adsorbent that exhibits a high efficiency for VOCs. After sufficient time for purging and trapping have been allowed, the trap is rapidly heated to thermally desorb the VOCs off of the adsorbent and directly into the GC. In this manner, VOCs are removed from the environmental sample without a matrix effect and the objectives of TEQA can be met.

Purge and trap is dynamic; the analyte is merely transferred from the sample to a organic polymeric matrix that exhibits a large surface area. One point of view assumes that static HS is merely a screen for dynamic HS (P&T) because MDLs for P&T are significantly lower than that for static HS. A second approach to screening for particular priority pollutant VOCs is called hexadecane screening. The logic as to what technique to use is depicted in the flowchart in Figure 3.9. The sample matrix plays a significant role as to whether one chooses static or dynamic HS techniques for the isolation and recovery of VOCs from environmental samples.

When analyzing environmental samples that are considered grossly contaminated, screening techniques are essential. Screening techniques serve to inform the analyst as to whether or not a dilution of the original is warranted. Laboratories that have both automatic static HS and automated P&T systems are more likely to use static HS for the initial screen, followed by P&T for the quantitative analysis. This is particularly true for labs that engage in EPA contract work. Labs that have either static HS or P&T, but not both, often elect to use hexadecane screening prior to the quantitative determination by either static HS or P&T. Labs that analyze predominantly drinking water sample most likely do not have a need to screen samples and usually proceed directly to the available determinative technique.

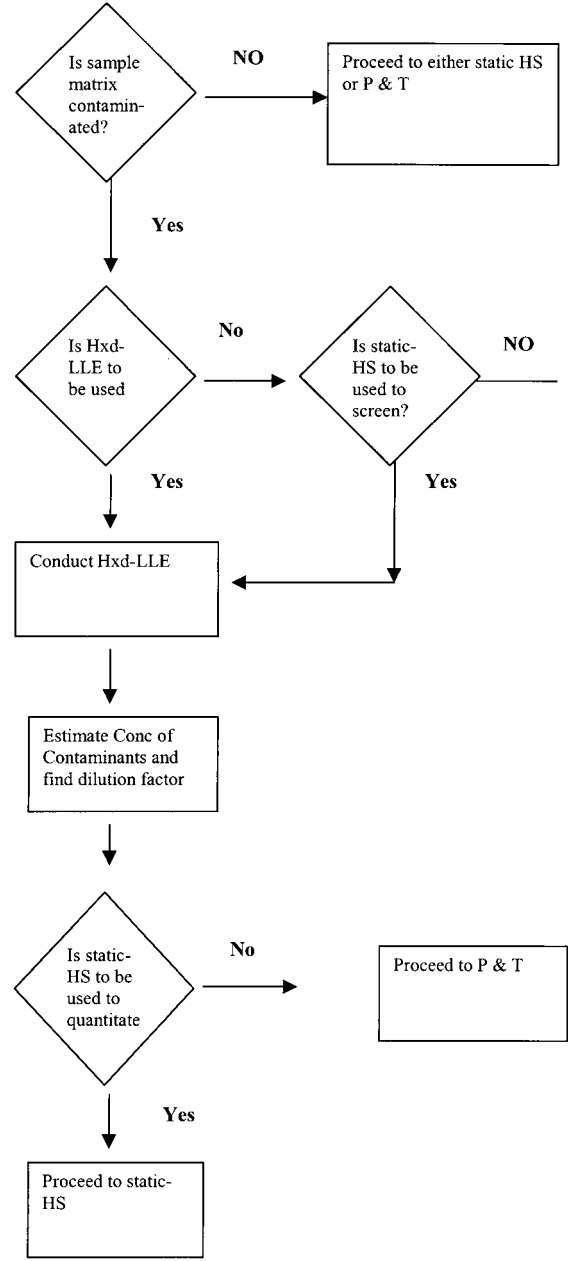


FIGURE 3.9 Flowchart for decisions involving screening of environmental samples for VOCs.

34. WHAT IS HEXADECANE SCREENING?

Hexadecane screening uses hexadecane (Hxd) as the extractant to perform an initial LLE of a water or wastewater sample that is suspected of containing appreciable levels of VOCs in a complex sample matrix. We thus refer to this technique with the abbreviation Hxd-LLE. Figure 3.10 is a gas chromatogram, GC-gram, of a groundwater sample that has been in contact with gasoline. This is a common occurrence in the environment because gasoline storage tanks are known to corrode with age and leak into the groundwater supply, thus contaminating sources of drinking water. The VOCs, commonly referred to as BTEX components, are clearly evident in the GC-gram. BTEX is an abbreviation for the aromatic VOCs benzene, toluene, ethylbenzene, and one or more of the three isomeric xylenes, ortho, para, and meta. A fourth component is more frequently showing up in groundwater samples. This compound is methyl-*t*-butyl ether (MTBE). MTBE is added to gasoline as an oxygenated hydrocarbon. A groundwater sample that exhibits a significant concentration of BTEX should be diluted with high-purity water. This diluted sample should be subsequently analyzed to quantitatively determine the concentration of BTEX and MTBE using either static HS or P&T. If a hydrocarbon of much lower molecular weight, such as *n*-hexane, were used to conduct the preliminary assessment of the degree of sample contamination, the instrument response due to the elution of *n*-hexane from the GC would interfere with one or more of the peaks due to the presence of BTEX. It is illustrative to compare the physical properties of *n*-hexane versus *n*-hexadecane. The following table compares the physical properties of both hydrocarbons:

Compound	MW	$T_b(^{\circ}\text{C})$	$\rho^{20^{\circ}\text{C}}(\text{g/mL})$	P'	Solution in water (%)
<i>n</i> -hexane	86	68.7	0.659	0.1	0.014
Hexadecane	226	287	0.773	0.5	—

It is important to use as high a purity of Hxd as can be obtained; otherwise, impurities in this extracting solvent might cause interferences with some VOCs. The absence of lower-molecular-weight impurities in Hxd early in the GC-gram greatly contributes to lowering MDLs. A GC that incorporates a flame ionization detector is usually designated as the Hxd-LLE screening instrument. A mini-LLE scale using a phase ratio, $\beta = V_o/V = 0.057$, such that approximately 35 mL of aqueous sample is extracted once into 2 mL of Hxd is a common technique. The limitations

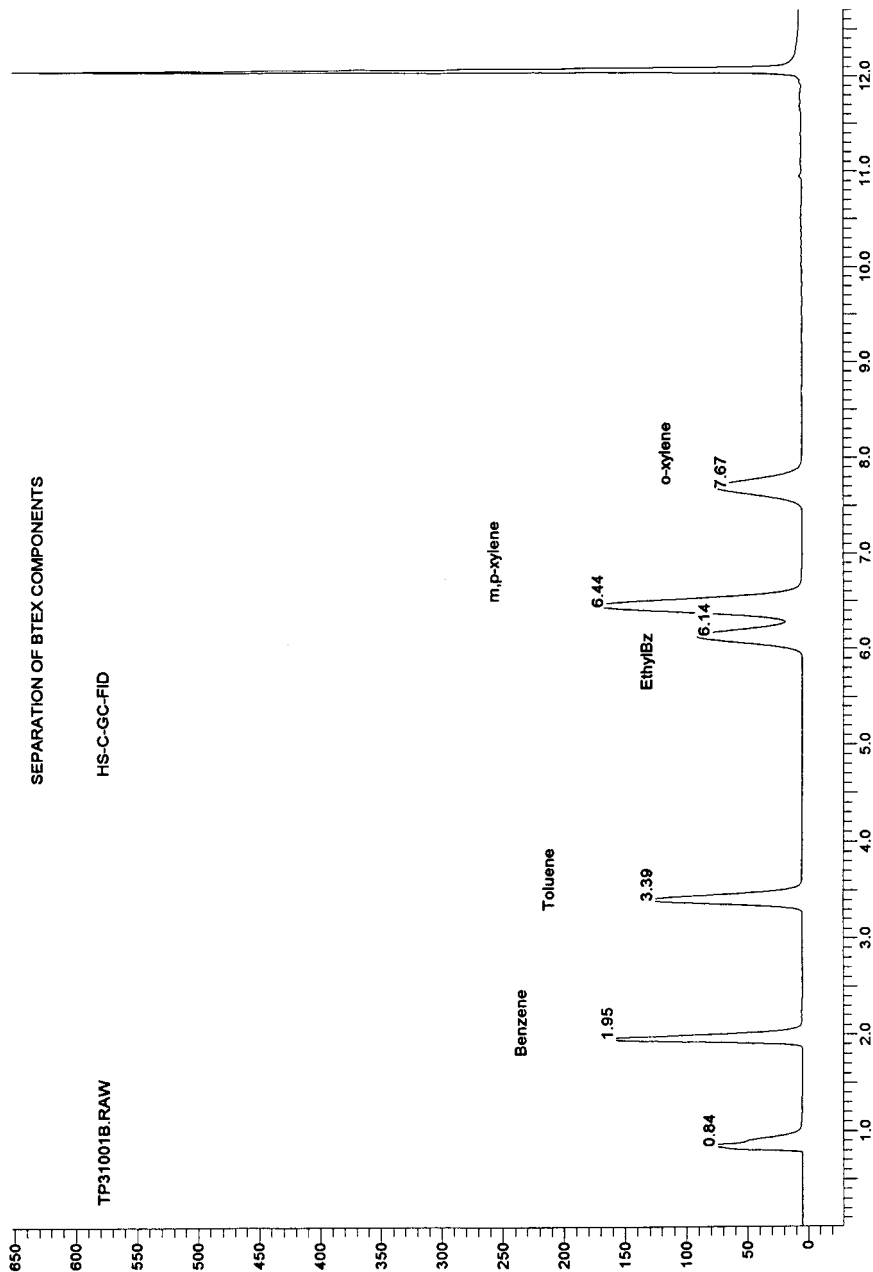


FIGURE 3.10 Capillary gas chromatogram for the separation of BTEX components in a water sample contaminated with gasoline.

to the effective use of Hxd-LLE lie in the nature of the environmental sample matrix. Samples such as wastewaters that are prone to cause emulsions prevent analysts from quickly injecting a 1- μ L aliquot of the extract. Common techniques for breaking emulsions include the following (35):

1. Adding salt to the aqueous phase
2. Using a heating-cooling extraction vessel
3. Filtering the emulsion through a glass wool plug
4. Filtering the emulsion through a phase-separation filter paper
5. Using a centrifuge
6. Adding a small amount of a different organic solvent

If this author is asked to estimate the concentration level of VOCs and static HS or P&T techniques are not available, he would employ Hxd-LLE! EPA Method 3820 from the SW-846 series is a useful starting starting point for this LLE technique. Seven different approaches to preparing environmental samples to determine VOCs are presented in SW-846. We will discuss each of these with an emphasis on the operational aspects.

35. WHAT ARE EPA'S APPROACHES TO TRACE VOCS?

Method 5000 in the SW-846 lists seven methods to prepare samples for the quantitative determination of VOCs. These methods reflect the different sample matrices involved. Water, soil/sediment, sludge, and waste samples that require analysis for VOCs are extracted and/or introduced into a GC or a GC-MS system by the various methods, as discussed below. The following methods are briefly introduced from an EPA point of view and the advantages / limitations from that viewpoint are discussed.

Method 3585 describes a solvent dilution technique using hexadecane followed by direct injection into a GC-MS instrument for the determination of VOCs in waste oils. Direct injection of an oil waste could lead to instrument-contamination problems. However, the method provides for a quick turnaround.

Method 5021 describes the automated static-HS technique. Static HS has been introduced in this book from a theoretical viewpoint. A soil sample is placed in a tared septum-sealed vial at the time of sampling. A matrix modifier containing internal and/or surrogate standards is added. The sample vial is placed into an automated equilibrium headspace sampler. The vial's temperature is elevated to a fixed value that does not change over time and the contents of the vial is mixed by mechanical agitation. A measured volume of headspace is automatically introduced into a GC or a GC-MS. The method is automated and downtime is minimal. However, the cost of the automated system is appreciable. Contamination of the instru-

ment is minimal. The MDL for this technique is significantly influenced by the choice of GC detector.

Method 5030 describes the technique of purge and trap for the introduction of purgeable organics into a GC or GC-MS. The method is applicable to aqueous samples such as groundwater, surface water, drinking water, wastewater, and water-miscible extracts prepared by Method 5035. An inert gas is bubbled through the sample and the VOCs are efficiently transferred from the aqueous phase to the vapor phase. The vapor phase is swept through a sorbent trap where the purgeables are trapped. After purging is completed, the trap is heated and backflushed with the inert gas to desorb the purgeables onto a GC column. P&T is easily automated and provides good precision and accuracy. However, the method is easily contaminated by samples that contain compounds that are present in the sample at the ppm level. Since P&T is an exhaustive removal of VOCs from the environmental sample, some argue that this technique offers the lowest MDLs. Again, the MDL would be strongly influenced by the choice of GC detector. Because this is the most commonly used method to determine VOCs, the method will be elaborated upon later.

Method 5031 describes an azeotropic distillation technique for the determination of nonpurgeable, water-soluble VOCs that are present in aqueous environmental samples. The sample is distilled in an azeotropic distillation apparatus, followed by direct aqueous injection of the distillate into a GC or GC-MS system. The method is not amenable to automation. The distillation is time-consuming and is limited to a small number of samples.

Method 5032 describes a closed-system vacuum distillation technique for the determination of VOCs that include nonpurgeable, water-soluble, volatile organics in aqueous samples, solids, and oily waste. The sample is introduced into a sample flask that is, in turn, attached to the vacuum distillation apparatus. The sample chamber pressure is reduced and remains at approximately 10 torr (the vapor pressure of water) as water is removed from the sample. The vapor is passed over a condenser coil chilled to a temperature of 10°C or less. This results in the condensation of water vapor. The uncondensed distillate is cryogenically trapped on a section of $\frac{1}{8}$ -in. stainless-steel tubing chilled to the temperature of liquid nitrogen (-196°C). After an appropriate distillation period, the condensate contained in the cryogenic trap is thermally desorbed and transferred to the GC or GC-MS using helium carrier gas. The method very efficiently extracts organics from a variety of matrices. The method requires a vacuum system along with cryogenic cooling and is not readily automated.

Method 5035 describes a closed-system P&T system for the determination of VOCs that are purgeable from a solid matrix at 40°C. The method

is amenable to soil/sediment and any solid waste sample of a consistency similar to soil. It differs from the original soil method (Method 5030) in that a sample, usually 5 g, is placed into the sample vial at the time of sampling along with a matrix-modifying solution. The sample remains hermetically sealed from sampling through analysis as the closed-system P&T device automatically adds standards and then performs the purge and trap. The method is more accurate than Method 5030 because the sample container is never opened. This minimizes the loss of VOCs through sample handling. However, it does require a special P&T device. Oil wastes can also be examined using this method.

Method 5041 describes a method that is applicable to the analysis of sorbent cartridges from a volatile organic sampling train. The sorbent cartridges are placed in a thermal desorber that is, in turn, connected to a P&T device.

36. WHAT IS THE P&T TECHNIQUE?

The P&T method to isolate, recover, and quantitate VOCs in various environmental sources of water has and continues to remain as the *premier technique* for this class of environmental contaminants. The technique was developed at the EPA in the early 1970s (36) and remains the method of choice, particularly for environmental testing labs that are regulatory driven. P&T has had its most success with drinking water samples when combined with gas chromatography and element-specific detectors. GC detectors will be discussed in Chapter 4. In this section, we will discuss the EPA methods that use the P&T technique to achieve the goals of TEQA. EPA Method 502.2 summarizes the method as follows (37):

Highly volatile organic compounds with low water solubility are extracted (purged) from the sample matrix by bubbling an inert gas through a 5 mL aqueous sample. Purged sample components are trapped in a tube containing suitable sorbent materials. When purging is complete, the sorbent tube is heated and back-flushed with helium to thermally desorb trapped sample components onto a capillary gas chromatography (GC) column. The column is temperature programmed to separate the method analytes which are then detected with a photoionization detector (PID) and an electrolytic conductivity detector (ElCD) placed in series. Analytes are quantitated by procedural standard calibration . . . Identifications are made by comparison of the retention times of unknown peaks to the retention times of standards analyzed under the same conditions used for samples. Additional confirmatory information can be

gained by comparing the relative response from the two detectors. For absolute confirmation, a gas chromatography/mass spectrometry (GC/MS) determination according to EPA Method 524.2 is recommended.

The classical purge vessel is shown in Figure 3.11. Usually, 5 mL of an environmental aqueous sample is placed in the vessel. The sample inlet utilizes a two-way valve. A liquid-handling syringe that can deliver at least 5 mL of sample is connected to this sample inlet and the sample is transferred to the purge vessel in a way that minimizes the sample's exposure to the atmosphere. Note that the incoming inert purge gas is passed through a molecular sieve to remove moisture. A hydrocarbon trap can also be inserted prior to the purge vessel to remove traces of organic impurities as well. The vessel contains a fritted gas sparge tube that serves to finely divide and to disperse the incoming purge gas. These inert gas bubbles from the purging provide numerous opportunities for dissolved organic solutes to escape to the gas phase. The acceptable dimensions for the purge device are such, according to EPA Method 502.2, that it must accommodate a 5-mL sample with a water column at least 5 cm deep. The headspace above the sample must be kept to a minimum of < 15 mL to eliminate dead-volume effects. The glass frit should be installed at the base of the sample chamber with dispersed bubbles having a diameter of < 3 mm at the surface of the frit.

Figure 3.11 also depicts a typical trap to be used in conjunction with the purge vessel. The trap must be at least 25 cm long and have an inside diameter of at least 0.105 in., according to Method 502.2. The trap must contain the following amounts of adsorbents:

- One-third of the trap is to be filled with 2,6-diphenylene oxide polymer, commonly called Tenax.
- One-third of the trap is to be filled with silica gel.
- One-third of the trap is to be filled with coconut charcoal.

It is recommended that 1 cm of methyl-silicone-coated packing be inserted at the inlet to extend the life of the trap. Method 5030B from the SW-846 series is more specific than Method 502.2 and recommends a 3% OV-1 on Chromosorb-W, 60/80 mesh, or equivalent. Analysts who do not need to quantitate dichlorodifluoromethane do not need to use the charcoal and can replace this charcoal with more Tenax. If only analytes whose boiling points are above 35°C are to be determined, both the charcoal and the silica gel can be eliminated and replaced with Tenax. The trap needs to be conditioned at 180°C prior to use and vented to the atmosphere instead of the analytical GC column. It is also recommended that the trap be reconditioned on a

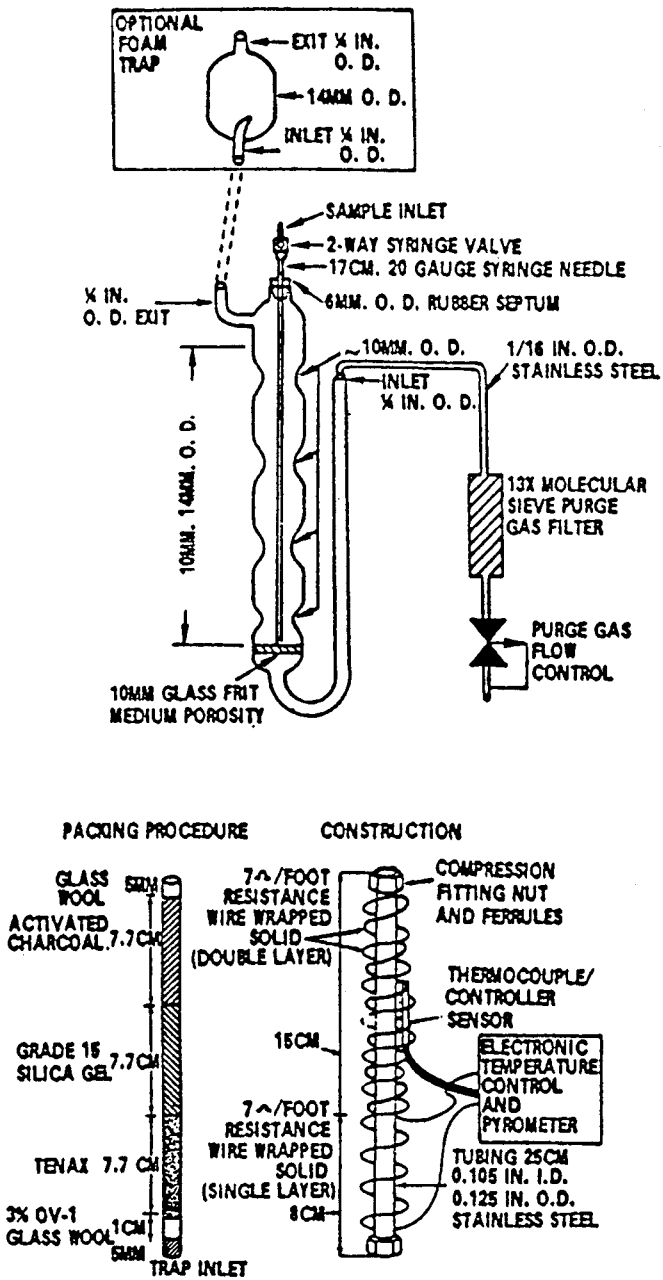


FIGURE 3.11 Schematic of a purge tube and trap configuration.

daily basis at the same temperature. Tenax is a unique polymer and offers the advantage that water is not trapped to any great extent.

Commercially available P&T units are fully automated and consist of a bank of purge vessels with switching valves that enable one vessel to be purged after another. Figure 3.12 is a schematic diagram that clearly depicts the purge, trap, and thermal desorption steps involved in this technique. A six-port valve, placed after the trap and interfaced to the injection port of a GC, provides the needed connection. Referring to Figure 3.12a, the P&T step is shown. Inert gas, usually helium, enters the purge vessel while the trap outlet is vented to the atmosphere. Meanwhile, GC carrier gas flows through one side of the six-port valve directly to the GC. The purge vessel and the trap are generally kept at ambient temperature. Some commercially available units provide for a heated purge vessel. Referring to Figure 3.12b, the direction of gas flow during the desorb step is illustrated. The valve is turned and inert gas enters and passes over the trap in a direction opposite to the P&T step. The trap is rapidly heated to the required final temperature and the trap outlet is directed to the injection port of the GC. GCs that are equipped with cryogenic cooling can deposit the VOCs from the trap to the GC column inlet as a plug. The GC can then be temperature programmed from the cryogenic temperature to the final temperature and, hence complete the gas chromatographic separation of all VOCs.

Operating conditions drawn from EPA Method 5030B for the P&T system differ slightly based on which determinative method is used and are presented in the following:

Analysis method	8015	8021 or 8260
Purge gas	N ₂ or He	N ₂ or He
Purge gas flow rate (mL/min)	20	40
Purge time (min)	15.0	11.0
Purge temperature (°C)	85	Ambient
Desorb temperature (°C)	180	180
Back-flush inert gas (mL/min)	20–60	20–60
Desorb time (min)	1.5	4

37. HOW DOES ONE GO ABOUT CONDUCTING THE P&T TECHNIQUE?

Let us assume that the decisions of whether or not to screen the sample prior to conducting P&T have been made and that we are ready to actually perform the technique. We have available in our laboratory organic-free water,

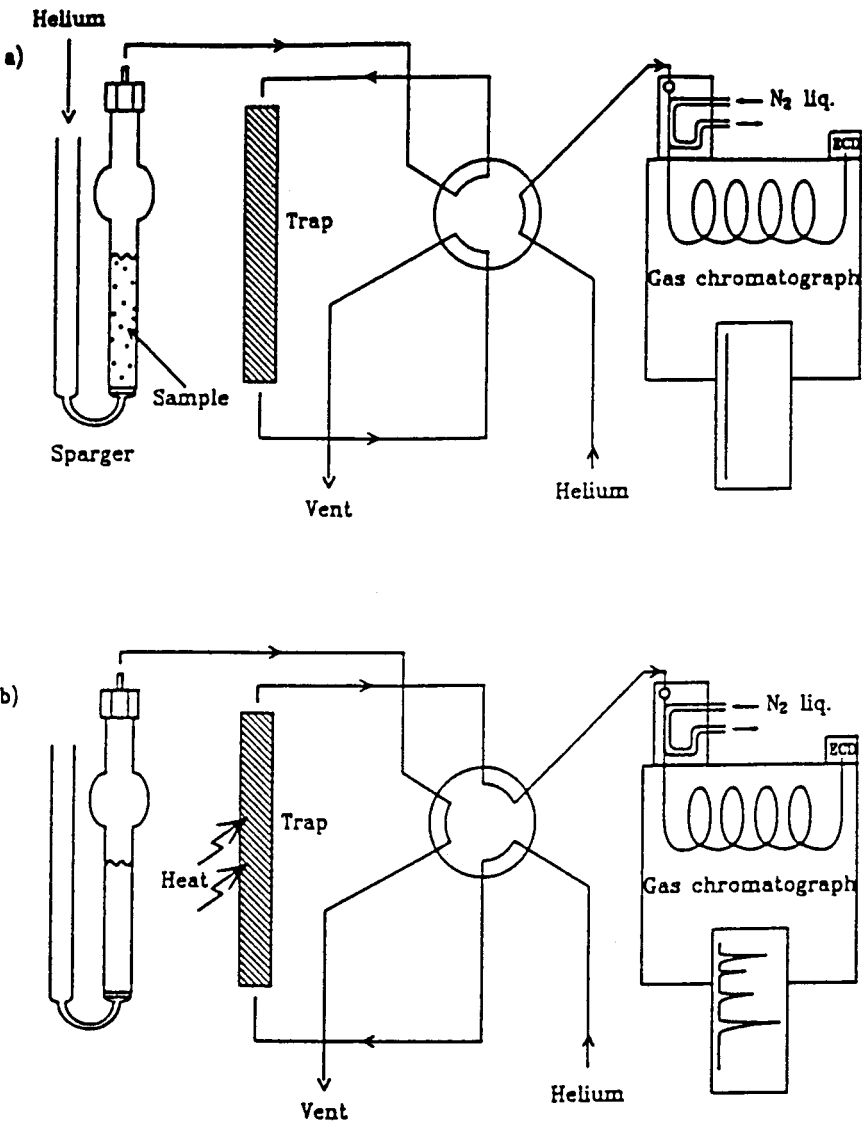


FIGURE 3.12 Schematics of the purge, trap, and thermal desorption for trace VOCs in water.

the determinative method has been defined, and we have reviewed EPA Method 5000 for guidance with respect to internal and surrogates standards. Environmental samples such as groundwater, drinking water, wastewater, and so forth have already been collected, stored in capped bottles with minimum headspace, free of solvent fumes, and stored at 4° C. If the sample was found to be improperly sealed, it should be discarded. All samples should be analyzed within 14 days of receipt at the laboratory. Samples not analyzed within this period must be noted and data are considered to represent a minimum value. This is the essence of the so-called *holding times*. Holding times, like detection limits that were discussed in Chapter 2, are a controversial topic between the regulatory agency and contracting laboratory. Using Method 5030B for guidance, the sequence of steps needed to carry out the method are as follows:

- Initial calibration: The P&T apparatus is conditioned overnight with an inert gas flow rate of at least 20 mL/min and the Tenax trap held at a temperature of 180°C. The P&T apparatus is connected to the GC and each purge vessel is filled to its total volume with organic-free water. Methanolic solutions containing VOCs at high enough concentration such that only microliter aliquots need to be added to the water in each purge vessel are added to a series of purge vessels. A blank and set of working calibration standards are prepared right in the purge vessels. Internal standards and surrogates are also added as their methanolic solutions as standard references in a manner similar to the analytes to be calibrated. Refer to Chapter 2 for a discussion of the various modes of instrumental calibration.
- Conduct the P&T for the set of blanks and working calibration standards.
- Initial calibration verification (ICV): Prepare one or more purge vessels that contain the ICV. Sometimes, it is advantageous to prepare ICVs in triplicate to enable a preliminary evaluation of the precision and accuracy of the calibration to be made. The ICV criteria are usually given in a determinative method such as in the 8000 series of SW-846. These criteria must be met before real samples can be run.
- Adjust the purge gas flow rate, referring to the guidance given in the above table.
- Sample delivery to the purge vessel: Remove the plunger from a 5-mL liquid-handling syringe and attach a closed syringe valve. Open the sample or standard bottle, which has been allowed to come to ambient temperature. Carefully pour the sample into the

syringe barrel to just short of overflowing. Replace the syringe plunger and compress the sample. Open the syringe valve and vent any residual air while adjusting the sample volume to 5.0 mL. This process of taking an aliquot destroys the validity of the liquid sample for future analysis. If there is only one sample vial, the analyst should fill a second syringe at this time to protect against possible loss of sample integrity. Alternatively, carefully transfer the remaining sample into a 20-mL vial and seal with zero headspace. The second sample is maintained only until such time when the analyst has determined that the first sample has been analyzed properly. In this way, the VOC content of the environmental sample is preserved during the transfer to the purge vessel.

A GC-gram from EPA Method 502.2 that depicts the separated peaks from both the PID and EICD detectors is shown in Figure 3.13. Both chromatograms are overlaid and show the different response for each chromatographically resolved peak between detectors.

38. DO ALL VOCs PURGE WITH THE SAME RATE?

No, they do not, and until recently, little was known about the kinetics of purging for priority pollutant VOCs using conventional P&T techniques. We describe the findings of Lin et al. (38), who demonstrated that first-order kinetics are followed for the removal of VOCs from P&T vessels. The authors make the point that because EPA methods such as 524.2 and 624 require the use of internal standards to obtain relative response factors, the percent purge efficiency is never considered. Some 28 priority pollutant VOCs were studied. Each VOC, dissolved in methanol, was spiked into high-purity water at concentration levels of 1 and 10-ppb with a 10-ppb internal standard. Spiked samples were purged for 11 min. A GC-MS was used and the absolute peak area for the characteristic primary ion was used to quantitate. Experimentally, samples were purged with helium for 11 min and GC-MS data were obtained. These purged samples were then purged a second time for the same 11-min duration under identical conditions. Peak areas were obtained in the same manner as for the first purge.

Let us begin to construct a mathematical view of the kinetics of P&T by first defining the purge ratio, P_i , as being the ratio of the mean GC-MS area count for the first purge to the mean GC-MS area count for the second purge according to

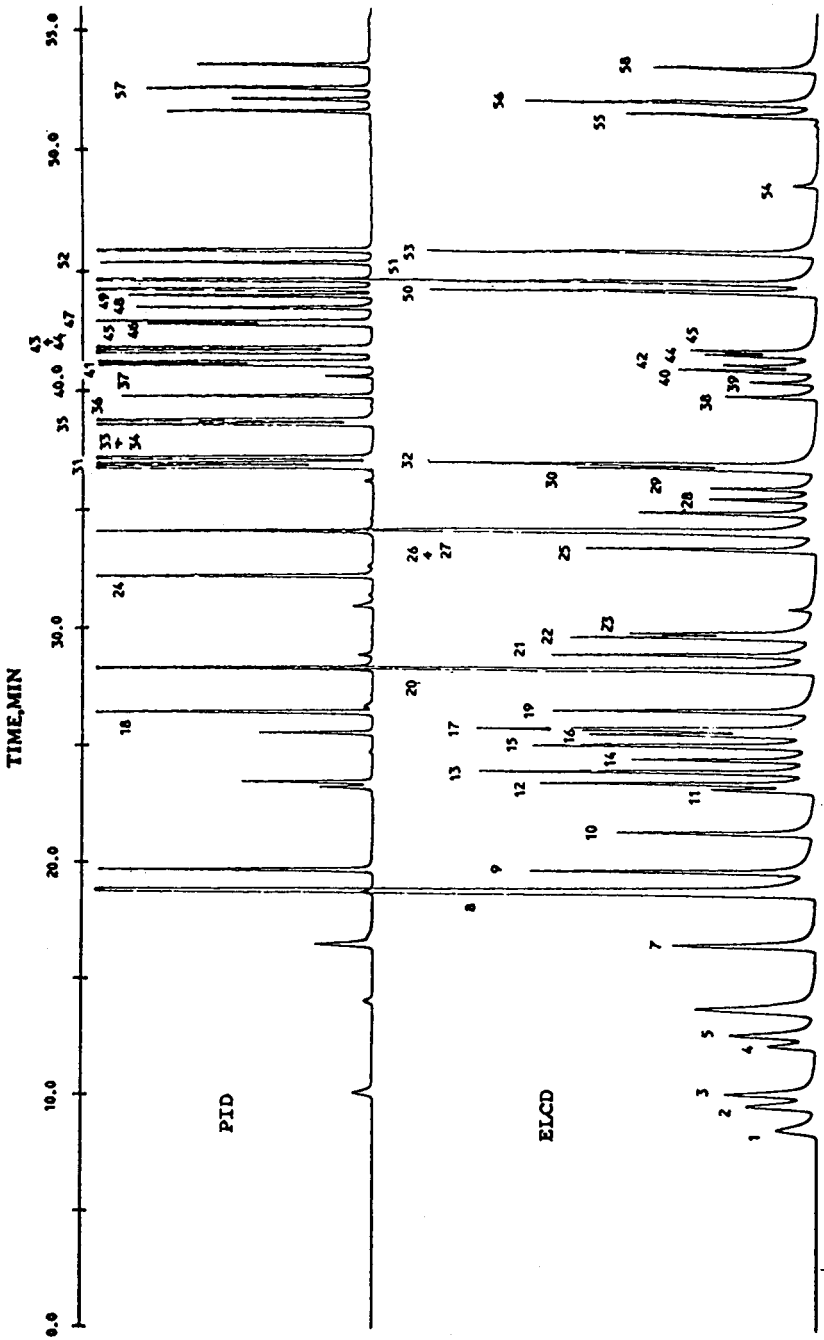


FIGURE 3.13 Capillary gas chromatogram for the separation of VOCs using EPA Method 502.2.

$$P_i = \frac{A_{i(\text{ave})}^1}{A_{i(\text{ave})}^2} = \frac{\sum_j^5 A_{ij}^1/5}{\sum_j^5 A_{ij}^2/5}$$

Each peak area for the i th analyte was obtained by averaging peak areas over j replicate purges with j taken from 1 to 5. A selected list of VOCs along with each VOC retention time, purge ratio, and coefficient of variation (refer to Chapter 2) from their work is given in the following table:

VOC	$t_R(\text{min})^a$	P_i	% RSD
Chloromethane	1.82	339	36
1,2-Dichloroethane	9.99	2.21	6
1,1,1,2-Tetrachloroethane	16.98	6.06	9
1,2,4-Trichlorobenzene	26.30	3.64	8
Acetone	4.30	1.05	2
2-Butanone	8.34	1.03	5

^a 30m $\times$ 0.53mm DB-624 (J & W Scientific), refer to EPA Method 524.2 for GC column temperature program and other GC conditions.

The low % RSDs, except for the very volatile chloromethane, indicates that P_i is constant over the range of concentrations studied. According to these authors, consistent values of P_i would indicate that purging VOCs from an aqueous solution can be mathematically described by first-order chemical kinetics. How fast any of the 28 VOCs studied are removed from a fixed volume of an aqueous sample such as groundwater in a conventional purge vessel is expressed as $-dC/dt$, where the negative sign indicates that the concentration of a VOC decreases with time. First-order kinetics suggests that this rate is directly proportional to the concentration of each VOC (39). Expressed mathematically,

$$\frac{dC}{dt} = -kC \quad (3.32)$$

where C is the analyte concentration at any time t and k is the first-order rate constant.

Differences in the magnitude of k depends on the chemical nature of the particular VOC and the degree of intermolecular interaction with the sample matrix—in this case, a relatively clean water sample. Equation (3.32) is first rearranged as

$$\frac{dC}{C} = -k dt$$

Integrating between the initial concentration C_1 and the concentration after the first purge, C_2 on the left side, while integrating between time $t = t_0$ and time $t = t_0 + \Delta t$ on the right side is shown below. Identical integration is also appropriate between C_2 and C_3 . Δt is the time it takes to purge the sample. We then have

$$\int_{C_1}^{C_2} \frac{dC}{C} = -k \int_{t_0}^{t_0 + \Delta t} dt$$

Evaluating the definite integrals for both sides of the equation yields

$$\ln(C_2 - C_1) = -k\Delta t$$

Utilizing a property of logarithms gives

$$\ln \frac{C_2}{C_1} = -k\Delta t$$

Expressed in terms of exponents,

$$\begin{aligned} \frac{C_2}{C_1} &= e^{-k\Delta t} \\ \therefore C_2 &= C_1 e^{-k\Delta t} \end{aligned} \quad (3.33)$$

Equation (3.33) implies that C_2 can be expressed in terms of C_1 , k , and Δt .

The experiment starts with each VOC at a concentration C_1 . After purge 1, each analyte is at concentration C_2 . After purge 2, each analyte is at concentration C_3 . This is depicted by:



Because VOCs are continuously removed from a fixed volume of aqueous sample, it is also true that

$$C_3 < C_2 < C_1$$

The peak area obtained after the first purge A^1 is proportional to the difference in concentration between C_1 and C_2 ; that is,

$$A^1 \propto C_1 - C_2$$

Substituting for C_2 using Eq. (3.33) gives

$$A^1 \propto C_1(1 - e^{-k\Delta t})$$

Likewise, the peak area obtained for each analyte after the second purge is proportional to the difference in concentration between C_2 and C_3 according to

$$A^2 \propto C_2 - C_3$$

Substituting for C_3 using Eq. (3.33) gives

$$A^2 \propto C_2(1 - e^{-k\Delta t})$$

The ratio of peak areas between the first and second purges for a given analyte, P , can be related to these differences in concentration as follows:

$$P = \frac{C_1 - C_2}{C_2 - C_3} = \frac{C_1}{C_2}$$

Substituting for Eq. (3.33) yields

$$P = \frac{C_1}{C_1 e^{-k\Delta t}}$$

This simplifies to

$$P = e^{k\Delta t} \tag{3.34}$$

Equation (3.34) suggests that P is always greater than 1 and is independent of the initial analyte concentration. The development of Eq. (3.34) has assumed first-order kinetics. The authors observed good precision over the 5 replicate experiments for all 28 VOCs studied, with the exception of the very volatile chloromethane.

If P is determined experimentally, by first obtaining the GC-MS peak area, A_1 , and then obtaining A_2 , then Eq. (3.34) can be solved for the first-order rate constant, k .

In addition, when $C_2 = \frac{1}{2}C_1$, the time it takes for one-half of the analyte to be removed can be found according to

$$\begin{aligned}
 \frac{C_1}{C_2} &= \frac{C_1}{\frac{1}{2}C_1} = 2 \frac{C_1}{C_1} = e^{kt^{1/2}} \\
 2 &= e^{k\eta^{1/2}} \\
 \ln 2 &= kt_{1/2} \\
 \therefore t_{1/2} &= \frac{1}{k} \ln 2
 \end{aligned} \tag{3.35}$$

Using Eq. (3.35), the $t_{1/2}$ values given for the three VOCs from the author's work are shown in the following table:

VOC	$t_{1/2}$ (min)
Chloromethane	1.3
1,1,1,2-Tetrachloroethane	4.2
Acetone	156

A purge time, $\Delta t = 11$ min is used in the indicated EPA methods. For hydrophobic VOCs, the $t_{1/2}$ seems to be too low, and for hydrophilic VOCs, the $t_{1/2}$ seems to be too high. Other generalizations emerge with respect to the chemical nature of the VOCs studied. The effect of deuterating 1,2-dichlorobenzene yields values for $t_{1/2}$ that are quite close to each other, 5.6 and 5.8 (1,2-dichlorobenzene- d_4) respectively. Differences in structural isomers such as 1,1,2,2 versus 1,1,1,2 tetrachloroethanes are reflected not only in their volatility in water but in their gas-phase stability of both neutral and ionic states. It would also appear that the choice of internal standard should reflect the kinetics of purging in addition to GC relative retention time. Choosing internal standards based on relative retention times has been the hallmark of most EPA methods. This work would suggest that $t_{1/2}$ also be taken into consideration when deciding on which internal standard to use when using P&T techniques.

We next move back to a consideration of semivolatile organics by introducing two alternative sample prep techniques that have been developed during the past 20 years and, at present, enjoy widespread popularity, supercritical fluid extraction for solids and solid-phase extraction for aqueous environmental samples.

Let us return to a consideration of the phase diagram shown in Figure 3.7 and consider a move both horizontal and vertical to the so-called supercritical region. We can accomplish similar analytical objectives to that addressed by ASE and that of interest to the practice of TEQA by choosing

a fluid whose supercritical pressures and temperatures can be reached in the laboratory. With the availability of carbon dioxide, an inexpensive fluid with relatively low supercritical fluid values (SFE), advanced.

39. HOW DO I GET STARTED DOING SFE?

In principle, you could merely replace the liquid and pump shown in Figure 3.16 and keep the high-pressure extraction cell and collection vial. You would need a means to both pressurize and to elevate the temperature of carbon dioxide. A critical temperature of 31.4°C with a critical pressure of 73 atm is needed to maintain CO₂ in a supercritical state. Consider again the phase diagram shown in Figure 3.7. Unlike liquids, if CO₂ is kept above 31.4°C, it cannot be liquified no matter how high the pressure. In this supercritical fluid state, the physical properties such as liquidlike density, intermediate diffusivity, gaslike viscosity, and gaslike surface tension provide a medium for rapid and selective extraction. SFE as an alternative to S-LSE was, in essence, “oversold” during the late 1980s and early 1990s. SFE does exhibit a matrix dependence. The use of organic modifiers has helped, however. Like ASE, SFE instrumentation is very expensive, and if only a few samples are to be prepared and analyzed for trace organics in environmental matrices, is it really worth the cost? In this section, we will discuss how SFE works and then, as we did for ASE, we will delve into some recovery studies.

40. WHAT LIQUIDS AND GASES CAN BE USED TO CONDUCT SFE?

Table 3.4 lists nine fluids together with their respective boiling points, their critical temperatures, critical pressures, and critical densities. From this list of nine, several can be eliminated as being either too hazardous or too corrosive to work with, such as ammonia, or as requiring too high a temperature, such as water and methanol. Carbon dioxide, nitrous oxide, xenon, ethane, ethylene, sulfur hexafluoride, and, very recently, fluoroform, CHF₃, have emerged as the likely candidates. Of these most suitable SF fluids, carbon dioxide emerges as the supercritical fluid of choice because CO₂ is nontoxic and inexpensive and exhibits a relatively low critical temperature and pressure. Its critical density is also appropriate. CO₂ is considered under supercritical fluid (SF) conditions as having a solvent polarity about that equal to hexane—in other words, nonpolar. The need to add polar, so-called “matrix modifiers” has led to increases in the percent recoveries of some of the more polar priority pollutants. The poor recovery of polar analytes from relatively nonpolar sample matrices has led to the notion of “inverse SFE” whereby the sample matrix can be completely

TABLE 3.4 Physico-chemical Properties for the Common Supercritical Fluids

Fluid	$T_{bp}(^{\circ}\text{C})$	$T_c(^{\circ}\text{C})$	$P_c(\text{atm})$	$\rho_f(\text{g/mL})$
CO_2	-78.5	31.3	72.9	0.448
NH_3	-33.3	132.4	112.5	0.235
H_2O	100.0	374.1	218.3	0.315
N_2O	-88.6	36.5	71.7	0.450
C_2H_6	-88.6	32.3	48.1	0.203
Xe	-108.1	16.6	58.4	1.10
CH_3OH	64.7	240.5	78.9	0.272
SF_6	63.8	45.5	7.1	0.740
	(sublimes)			
C_2H_4	-103.7	9.2	49.7	0.218

removed by SFE while the more polar analyte of remains. This has much more relevance to pharmaceutical analysis rather than to TEQA because sample matrices tend to be more polar than the analyte of interest. In the determination of a polar ingredient in an ointment, the sample matrix is extracted by the relatively nonpolar CO_2 leaving the polar-active ingredient in the SFE extraction vessel. Chester has recently reviewed the eight most likely candidates for use as supercritical fluid chromatography (SFC); this information is also pertinent to SFE (40).

41. WHAT OTHER PHYSICO-CHEMICAL PROPERTIES ARE OF INTEREST IN SFE?

Supercritical fluids begin to exhibit significant solvent strength when they are compressed to liquidlike densities. Figure 3.14 shows how the density of a pure SF changes as the SF is is compressed. The greatest change in SF density, ρ , is seen to occur in the region near to its critical point. An increase in the density of a supercritical fluid has been shown to increase the amount of a chemical compound that will dissolve in the SF (41). Certain other physico-chemical properties give SFs advantages with respect to SFE as well. The more a SF is compressed, the more liquidlike the fluid becomes. Very high-density SFs (i.e., $P \gg P_c$) are very liquidlike fluids. Very low-density SFs, where $T > T_c$ and $P \sim P_c$, are very gaseouslike. The three most useful physico-chemical properties of fluids, density, viscosity, and diffusion coefficient, are listed for gases, SFs, and liquids in Table 3.5. Interpretation of Table 3.5 clearly shows how SFs are intermediate between gases and liquids with respect to all three properties. It is not surprising that SFs

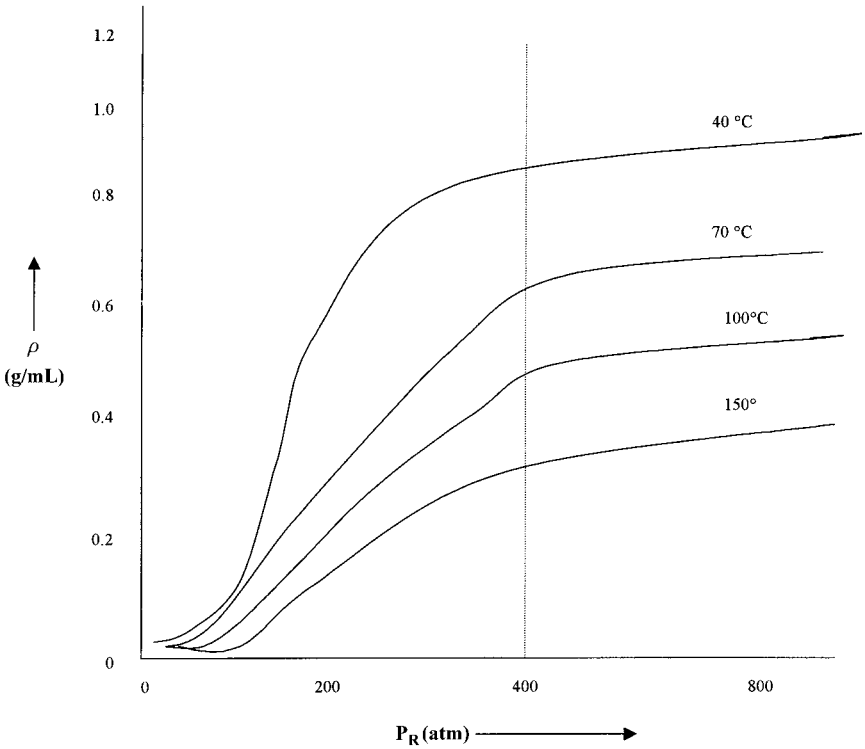


FIGURE 3.14 Influence of compressed gas pressure on the gas density of a supercritical fluid.

are denser than gases and, in turn, less dense with respect to liquids. SFs are more viscous than gases, yet less viscous by one order of magnitude than liquids. Diffusion coefficients are more important in considerations of SFC; however, it is interesting to compare the range of values of D for SFs versus liquids. SFs diffuse over 100 times faster than liquids.

TABLE 3.5 Typical Ranges of Densities (ρ), Viscosities (η), and Diffusion Coefficients (D) for Gases, Supercritical Fluids, and Liquids

Fluid	ρ (g/mL)	η (poise)	D (cm ² /s)
Gas	0.001	$0.5\text{--}3.5 (\times 10^{-4})$	0.01–1.0
SF	0.2–0.9	$0.2\text{--}1.0 (\times 10^{-3})$	$0.3\text{--}1.0 (\times 10^{-3})$
Liquid	0.8–1.5	$0.3\text{--}2.4 (\times 10^{-2})$	$0.5\text{--}2.0 (\times 10^{-5})$

The need to add organic modifiers to the supercritical CO_2 implies that a second component be considered from the perspective of the Gibbs Phase Rule. For two components, the number of degrees of freedom, f , is found according to $f = 4 - p$. Hence, for a one-phase region in the phase diagram where $p = 1$, three degrees of freedom are needed. In general, in addition to pressure and temperature, composition becomes this third degree of freedom. Therefore, phase diagrams must be viewed in three dimensions! To facilitate a two-dimensional view, in which regions of identical temperature can be plotted, so-called isotherms can be shown. Figure 3.15 is a three-dimensional representation of a pressure–temperature–composition (P – T – X) plot for a binary mixture. Figure 3.15 has been labeled to show the extremes of the composition axis where, using the example of a CO_2 /MeOH binary mixture, we start with pure CO_2 on one end and pure methanol on the other. The liquid–vapor equilibrium line is drawn for 0% MeOH (100% CO_2) and for 100% MeOH (0% CO_2). The dashed line connects the locus of mixture critical points. The significance of P – T – X plots for binary SF mixtures is that for values of P , T and X that reside above the curve, only one phase exists. For P , T , and X values that reside below the curve, two phases are possible depending on the composition (40). If 10% MeOH is

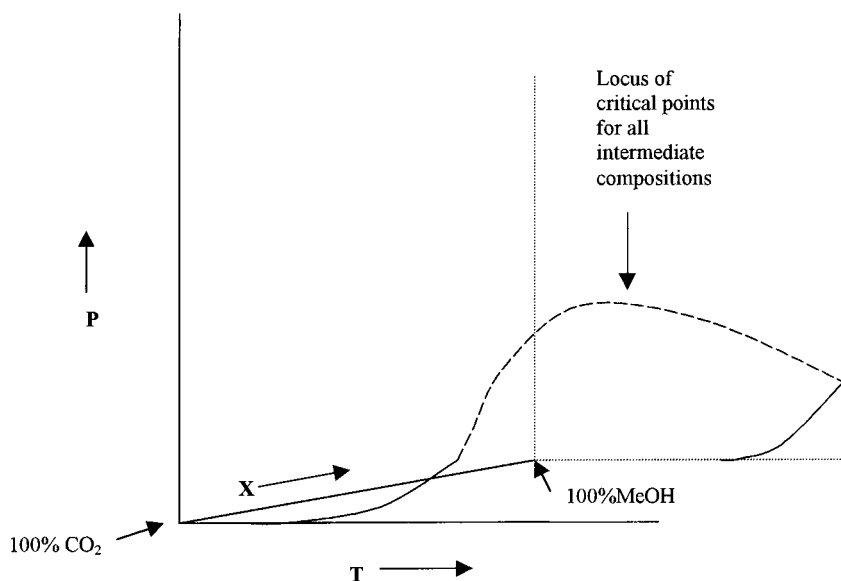


FIGURE 3.15 Three dimensional P – T – X (composition) plot for a binary mixture.

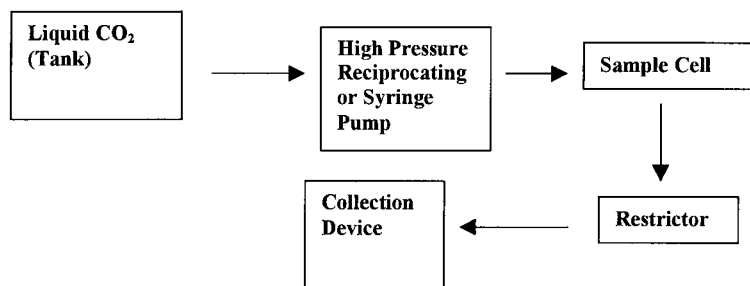


FIGURE 3.16 Schematic of principal components of an SFE instrument.

added as an organic modifier when using carbon dioxide as the SF, this plot is useful in knowing what conditions need to be reached to keep the SFE in one phase.

42. OF WHAT DOES SFE INSTRUMENTATION CONSIST?

A block diagram of the principal components of a SFE instrument is shown in Figure 3.16. It must be recognized that SFE–SFC-grade CO₂ requires a purity greater than 99.999%. The SF delivery system can be either a high-pressure reciprocating pump; or a high-pressure syringe pump. One's preference as to which type of pump to use depends on choice and cost considerations. It is necessary to cool the pump head when using a reciprocating pump. This need for cooling is unnecessary when using a syringe pump because CO₂ is liquefied by pressure not temperature. A syringe pump also eliminates the inconsistencies in repressurization that might be present in a reciprocating pumps, in other words, it provides a pulseless flow of CO₂. A syringe pump, however, is limited in capacity and must be refilled while a reciprocating pump draws continuously from a large reservoir. A second reciprocating pump is needed if the addition of an organic modifier is needed. This second pump does not need to be cooled. There have been other designs discussed in the literature as well, necessitated by the high cost of having to cool reciprocating pumps (42).

The sample cell or vessel is usually made of stainless steel or some other inert material and ranges in size from 150 μ L to 50 mL. It is most important that the sample vessels are rated for the maximum working pressure of the system. Cell designs are either of a flow-through (dynamic) or headspace sampling (static) type. Flow-through cells enable the SF to continuously pass through the sample. Static headspace sample cells enable the SF to surround the sample while the headspace above the sample is with-

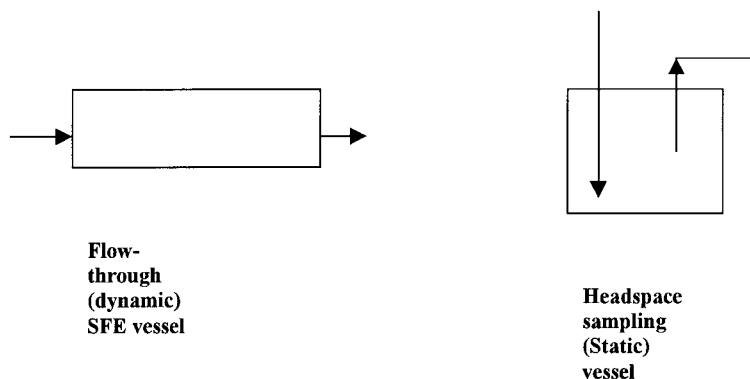


FIGURE 3.17 Schematic of flow-through and headspace sampling types of SFE sample cells.

drawn. Headspace sampling tends to be used for large samples, typically 40 mL. Figure 3.17 is a schematic showing the fundamental difference between these two cell designs (42).

The restrictor or depressurization step is very important and, over the years, has been the Achilles's heel of SFE. Plugging of restrictors was a major problem when this device consisted of an unheated fused-silica capillary tube. Restrictor problems have been minimized or even eliminated with the development of automated, variable restrictors (43). Variable restrictors use position sensors, motorized gears, and feedback from the CO_2 pump to control the flow setpoint and make flow adjustments. The restrictor is responsible for maintaining the pressure of the SF above its P_c . The restrictor induces a back-pressure in the sample cell. For a CO_2 -based SFE, the pump delivers the CO_2 in liquid form at a constant flow rate and SF conditions are established in the heated extraction chamber. The smaller the restrictor orifice, the higher the back pressure in the line and the greater the mass flow rate. The final part of SFE instruments is the collection device. Two approaches have been developed for this aspect of SFE instruments, on-line and off-line. On-line SFE refers to the coupling of the analyte-containing SF to a chromatographic separation system and abbreviations are used to indicate which determinative technique is interface. For example, SFE-GC implies that supercritical fluid extraction has been interfaced to a gas chromatograph. Off-line SFE refers to the stand-alone instrument whose block diagram is shown in Figure 3.16.

The collection device stores the extracted analyte in one of three ways: (a) via thermal trapping, (b) by sorbent trapping, or (c) by solvent trapping.

Thermal trapping is the simplest experimentally and merely involves directing the depressurized SFE effluent into a cooled vessel. Moderately volatile analytes are known to be lost using this type of SFE trapping; therefore, use of thermal trapping is limited to semivolatile and nonvolatile organic analytes. Sorbent trapping incorporates a solid adsorbent whose surface is capable of removing the extracted analytes from the SF effluent. The sorbed analytes are removed from the adsorbent by elution with a solvent. Sorbent trapping affords the opportunity to incorporate a degree of analyte selectivity either by selecting an appropriate sorbent or by the choice of elution solvent. Commercially available chemically bonded silicas have been used with success in sorbent trapping. We will have much more to say about the use of these materials with respect to the sample prep technique called solid-phase extraction later. Solvent trapping is the most widely used method of trapping analytes from the extracted SF effluent and merely involves depressurizing the SF directly into a small vial containing a few milliliters of liquid solvent. Hawthorne reports a tendency toward higher percent recoveries for sand that has been spiked with selected semivolatile organics such as phenol, nitrobenzene, and benzo[a]pyrene with a change in the chemical nature of the collection solvent. The following solvents were placed in a collection vial and the order in terms of a % collection efficiency was hexane < methanol < methylene chloride < methylene chloride kept at 5°C (by using a heating block) (44).

It has been well established that SF CO₂ is not a good extractant for polar analytes. In addition, some analyte–matrix interactions are sufficiently strong to yield low percent recoveries. The recovery of three PCB congeners were compared against Soxhlet extraction while using four different SFs, including SF CO₂ modified with methanol, are shown in Table 3.6 (44). The results show that the more polar SFE (CO₂/MeOH) yields as good a recovery as CHClF₂ whose dipole moment is 1.4 Debyes. Pure CO₂ has a dipole moment of 0.0 Debyes and the addition of a polar modifier like MeOH serves to increase the dipole moment of the SF fluid. This author is not

TABLE 3.6 Comparison of Soxhlet Extraction and Different SFE Fluids for the Recovery of PCB Congeners from River Sediment

PCB congener	Soxhlet	CHClF ₂	N ₂ O	CO ₂	CO ₂ /MeOH
2, 2', 3, 5'	1070	1158	497	470	855
2, 3', 4, 4', 5'	510	631	319	382	482
2, 2', 3, 4', 5, 5'	160	166	145	144	168

Source: Ref. 44.

aware if there are any published dipole moment values for polar modified SF CO₂.

43. IS THERE AN EPA METHOD THAT REQUIRES SFE AND IF SO, PLEASE DESCRIBE?

Yes there is; it is a recently developed method in the SW-846 series of methods that are most applicable to solid waste samples. It is Method 3562 titled “SFE of Polychlorinated Biphenyls (PCBs) and Organochlorine Pesticides (OCs)” (45). Twelve specific PCB congeners of either the tri-, tetra-, penta-, hexa-, or heptachlorobiphenyl variety have been studied in this method. Fourteen of the priority pollutant OCs from aldrin through to heptachlor epoxide have also been studied in this method. SFE is used to extract these analytes from soils, sediments, fly ash, solid-phase extraction media, and other solid materials that are amenable to extraction with conventional solvents. The uniqueness of this method is that it is possible to achieve a class separation (i.e., PCBs from OCs) by differences in SFE conditions. Samples to be analyzed for PCBs are subjected to a 10-min static extraction, followed by a 40-min dynamic extraction. Samples for OCs are subjected to a 20-min static extraction, followed by a 30-min dynamic extraction. The analyst must demonstrate that the SFE instrument is free from interferences. This is accomplished by extracting method blanks and determining the background contamination or lack thereof. The method cautions the user about carryover. Reagent blanks should be extracted immediately after analyzing a sample that exhibits a high concentration of analytes of interest. This method does not use a polar organic modifier. Referring again to the schematic of SFE instrumentation, Figure 3.16, Method 3562 specifies the nature of the extraction vessel, restrictor, and collection device. Vessels must be stainless steel with end fittings with 2- μ m frits. Fittings must be able to withstand pressures of 400 atm (5878 psi). The method was developed using a continuously variable nozzle restrictor. Sorbent trapping is the collection device used in the method. Florisil with a 30–40 μ m particle diameter is recommended for PCBs, whereas octadecyl silane is suggested to trap OCs. Solvent trapping is also suggested, with a cautionary remark concerning the loss of volatile analytes. The method requires the use of internal standards and surrogate standards. For sorbent trapping, *n*-heptane, methylene chloride, and acetone are recommended. The method also requires that a % dry weight be obtained for each solid sample. The weight loss that accompanies keeping 5–10 g of the sample in a drying oven overnight at 105°C is measured. The sample may need to be ground to ensure efficient extraction. To “homogenize” the sample, at least 100 g of ground sample is mixed with an equal

volume of CO₂ solid “snow” prepared from the SFE CO₂ solid. This mixture is to be placed in a food-type chopper and ground for 2 minutes. The chopped sample is then placed on a clean surface and the CO₂ solid “snow” is allowed to sublime away. Then, 1–5 g of the homogenized sample is weighed and placed in the SFE vessel. If the samples are known to contain elemental sulfur, elemental copper (Cu) powder is added to this homogenized sample in the SFE vessel. No adverse effect from the addition of Cu was observed and EPA believes that finely divided Cu may enhance the dispersion of CO₂. To selectively extract PCBs, the following SFE conditions using SF CO₂ are recommended:

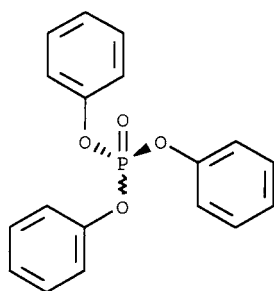
Pressure: 4417 psi
Temperature: 80°C
Density: 0.75 g/mL
Static equilibration time: 10 min
Dynamic extraction time: 40 min
SFE fluid flow rate: 2.5 mL/min

To extract OCs, the recommended SFE conditions are as follows:

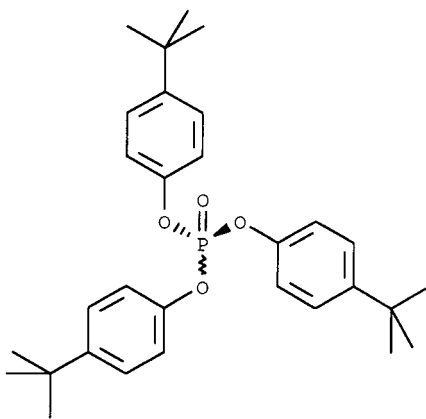
Pressure: 4330 psi
Temperature: 50°C
Density: 0.87 g/mL
Static equilibration time: 20 min
Dynamic extraction time: 30 min
SFE fluid flow rate: 1.0 mL/min

44. HAVE COMPARATIVE STUDIES FOR SFE BEEN DONE?

Yes, there have been comparative studies in which the percent recovery has been measured using not only SFE but also ASE and comparing these results to percent recoveries from the more conventional Soxhlet extraction (S–LSE). Generally, these studies are done on certified reference samples or on grossly contaminated samples. We will discuss two studies from the recent analytical chemistry literature. The first study compared the efficiencies of SFE, high-pressure solvent extraction (HPSE), to S–LSE for removal of nonpesticidal organophosphates from soil (46). HPSE is very similar to accelerated solvent extraction; however, we will reserve the acronym ASE for use with the commercial instrument developed by Dionex Corporation. HPSE as developed by these authors used parts available in their laboratory.. The authors compared S–LSE with SFE and HPSE for extraction of tricresyl phosphate (TCP) and triphenyl phosphate (TPP) from soil. Molecular structures for these two substances are as follows:



Triphenyl phosphate



Tricresyl phosphate

Ortho, meta, and para substitution within the phenyl rings can lead to isomeric TCPs. Aryl phosphate esters are of environmental concern due to their widespread use and release. They have in the past been used as fuel and lubricant additives and as flame-retardent hydraulic fluids. Widespread use of TCP associated with military aircraft have led to contamination of soil at U.S. Air Force (USAF) bases. TCP-contaminated soil samples were obtained from a USAF site. The % water was determined. Spiked soil samples were prepared by adding an ethyl acetate solution containing TPP, TCP, to 500 g of a locally obtained soil. This soil was placed in a rotary evaporator whereby the analytes of interest could be uniformly distributed throughout the soil. Methanol was added as the polar modifier directly into the soil in the SFE extraction chamber. It was deemed important to add methanol because TCP and TPP are somewhat polar analytes. The actual SFE consisted of 10 min of static SFE followed by 20 min of dynamic SFE. The sample, which consisted of 1.5 g of soil with 1.0 mL of sand, was placed in the bottom of the SFE vessel. A sorbent trapping technique using glass beads with subsequent washing with ethyl acetate was used to recover the analytes. The equivalent of ASE was conducted in this study, not with a commercial instrument as discussed earlier but with a combination of a commercially available SFC syringe pump (Model SFC-500 from Isco Corp.) and a gas chromatographic oven (Model 1700 GC from Varian Associates). We will use the author's abbreviation for high-pressure solvent extraction (HPSE). A series of SFE-related method development studies were first undertaken to optimize the effect of the volume of methanol and the effect of temperature and of pressure on percent recoveries of TCP from native soil. In a similar manner, extractions

conditions for HPSE were optimized by varying temperature and pressure. Optimized conditions arrived at were the following:

SFE: 80°C, 510 atm, and 1250 μL MeOH

HPSE: 100°C and 136 atm

The determinative technique was capillary gas chromatography equipped with a nitrogen–phosphorus detector. The authors found the percent recoveries to be quite close to S-LSE while reporting that considerable time and solvent consumption could be saved. Next, we consider a second comparative study.

Heemken and co-workers in Germany reported on percent recovery results for the isolation and recovery of PAHs and aliphatic hydrocarbons from marine samples (47). Results were compared using accelerated solvent extraction, ASE, SFE, ultrasonication (U-LSE), methanolic saponification extraction (MSE), and classical Soxhlet (S-LSE). Both ASE and SFE compared favorably to the more conventional methods in terms of a relative percent recovery against the conventional methods, so that extracted analytes from ASE and SFE were compared to the same extracted analytes from S-LSE and U-LSE. Relative percent recoveries ranged from 96% to 105% for the 23 two, through seven (coronene) fused ringed PAH and methyl-substituted PAHs. To evaluate the percent recoveries, the authors defined a bias, D_{rel} , according to

$$D_{\text{rel}} = \frac{X_1 - X_2}{X_1} \times 100\%$$

where X_1 and X_2 are the extracted yields for one method versus another. For example, a summation of the amount of nanograms extracted for all 23 PAHs using SFE was 34,346 ng, whereas for S-LSC, it was 33,331 ng. Using the above equation gives a bias of 3.0%. Table 3.7 gives values for D_{rel} for PAHs for (a) SFE versus either S-LSE or U-LSE and (b) ASE versus either S-LSE or U-LSE. Three different environmental solid samples were obtained. The first was a certified reference marine sediment sample obtained from the National Research Council of Canada. The second sample was a suspended sediment obtained from the river Elbe in Germany using a sedimentation trap. This sample was freeze-dried and homogenized. The third sample was a suspended sediment obtained from the river Weser and collected via a flow-through centrifuge. This sample was air-dried and had a water content of 5.3%. Values of D_{rel} were all within 10% among the four methods shown in Table 3.7 and it is safe to conclude that it makes no difference whether SFE or ASE, or S-LSE or U-LSE is used to extract PAHs from the three marine sediment samples. The other criteria used to

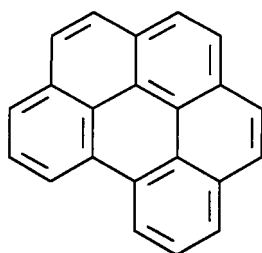
TABLE 3.7 Values of Bias D_{rel} for PAHs for Various Sample Preparation Methods

Sample	SFE vs S-LSC	SFE vs U-LSC	ASE vs S-LSC	ASE vs U-LSC
Certified Reference Marine Sediment	3.0	4.7	0.3	8.3
Freeze-dried homogenized suspended particulate matter	5.0	N	2.4	N
Air-dried- suspended particulate matter	-3.8	N	N	N

Note: N = not investigated.

compare different sample preparation methods is that of precision. Is SFE and ASE as reproducible as are S-LSE and U-LSE? For the certified reference standard, an overall relative standard deviation for PAHs was found to be 11.5%. For the first sediment, a RSD was found to range from 3.4% to 5.0% and for the second sediment, a RSD was found to range from 2.7% to 7.5%. Let us now consider the isolation and recovery of one priority pollutant PAH from this work.

Benzo(*g,h,i*) perylene, BghiP, is a five-membered fused phenyl ring PAH of molecular weight 277 and could be considered quite nonpolar. Its molecular structure is as follows:



Benzo(*g,h,i*) perylene

Shown in Table 3.8 are the yields of analyte in amount of nanograms ng BghiP per gram of dry sediment. Also included are yields from the MSE approach. MSE as defined in the paper is essentially a batch LLE in which

TABLE 3.8 Recovery of BghiP in Amount of ng BghiP/g Sediment Using Five-Sample Preparation Methods

Sample	SFE	ASE	S-LSE	U-LSE	MSE
Certified Reference Marine Sediment 1726 ± 720	1483(5.6) <i>n</i> = 6	1488(7.4) <i>n</i> = 6	1397(3.9) <i>n</i> = 3	1349(3.8) <i>n</i> = 3	1419(3.4) <i>n</i> = 3
Free-dried homogenized suspended particulate matter, I	147(2.3) <i>n</i> = 6	156(1.6) <i>n</i> = 3	207 <i>n</i> = 1		180(1.6) <i>n</i> = 3
Air-dried suspended particulate matter, II	122.5(1.2) <i>n</i> = 3		129.1(0.4) <i>n</i> = 3		127.2(0.1) <i>n</i> = 6
II, nondried, 56% water	35(9.0) <i>n</i> = 3	32(14.1) <i>n</i> = 3			98(0.6) <i>n</i> = 3
II, nondried, anhydrous sodium sulfate	96(3.7) <i>n</i> = 6				

Note: *n* is the number of replicate extractions. The number in parenthesis is the relative standard deviation expressed as a percent.

the sediment is refluxed in alkaline methanol followed by dilution with water and then extracted into hexane. The yield of BghiP is similar for all five sample prep methods as reported by the authors. The other significant outcome was to show that for a nondried marine sediment, the addition of anhydrous sodium sulfate increased the yield of BghiP from 35 to 96 ng and this result came close to that obtained by the MSE approach. One other emerging sample prep technique for solid matrices is worth mention—microwave-assisted extraction (MAE).

45. WHAT IS MICROWAVE-ASSISTED EXTRACTION OF ORGANICS FROM SOLID MATRICES?

One should not discuss SFE, ASE, S-LSE, and U-LSE without including a relatively recent technique that is being applied to solid matrices, called MAE. MAE is well established with respect to inorganic elemental analysis, a topic that will be discussed in the section on sample prep techniques

for inorganics. According to Lesnik, MAE is one of the “green” sample prep methods that “provide rapid, rigorous extraction procedures for the target analytes with minimal solvent use” (48). The other green methods include ASE, SFE for solid matrices, and solid-phase extraction for aqueous matrices. We will have much more to say about solid-phase extraction later. The term “microwave heating” conjures up what many of us do every single day in our kitchen (i.e., apply microwave energy to warming up food). MAE enables the temperature of a liquid–solid extraction to be elevated to 100–115°C and an increase in pressure in a closed vessel to between 50 and 175 psi (48). There is great danger in merely placing a soil sample to which an organic solvent such as hexane has been added and heating this in a microwave oven, because hexane is flammable. However, hexane itself, being nonpolar, does not heat when exposed to a microwave field (49). What is done is to add a chemically inert fluoropolymer insert filled with carbon black, a strong microwave absorber. Safety considerations therefore demand closed vessel and oven technology that is designed to prevent explosions and escape of toxic fumes. Thus, acquisition of microwave oven and accessory technology like SFE and ASE can be quite expensive.

46. HAVE THERE BEEN STUDIES APPLYING MAE TO ENVIRONMENTAL SAMPLES?

Yes, there have been. We will discuss the findings of Lopez-Avila and co-workers who report on their findings as part of an ongoing EPA research program. This program is designed to evaluate sample preparation techniques that (a) prevent or minimize pollution in analytical laboratories, (b) improve target analyte recoveries, and (c) reduce sample preparation costs (50). The nature and types of organic compounds that were spiked into soil include (a) semivolatiles such as PAHs, polychlorinated phenols, and phthalate esters, (b) organochlorine pesticides (OCs) and polychlorinated biphenyls (PCBs), and (c) organophosphorous pesticides (OPs). Some of the spiked soil was also aged before it was analyzed. Seventy-nine of the 95 semivolatile organics that were spiked into topsoil were isolated and recovered within the range 80–120 %. The recoveries of 14 compounds were below 80%. Upon spiking the topsoil and allowing a 24-h aging at 4°C and adding water to the spiked soil to ensure good mixing of the target compounds with the matrix, only 47 compounds had recoveries between 80% and 120%. In summary, the authors report that the MAE technique performed adequately for the semivolatiles listed in what was, at the time, EPA Method 8250, which is currently referred to as 8270C in revision 3, December 1996, to the third edition of SW-846. Representative

OCs, PCBs, and OPs were also adequately isolated and recovered using MAE. A second successive extraction was found not to be necessary. Reduced solvent consumption, 30 mL in MAE versus 300 mL for S-LSC, and reduced sample prep times were considered the main advantages of this alternative technique.

This completes our digression into alternative sample prep techniques for solid matrices. We now return to aqueous samples and entertain a somewhat detailed discussion of the prime alternative to liquid–liquid extraction that emerged over 20 years ago, namely liquid–solid extraction using chemically bonded silica gel, commonly called solid-phase extraction.

47. WHAT IS SOLID-PHASE EXTRACTION?

Solid-phase extraction, commonly abbreviated SPE, as a means to prepare environmental samples is a relatively recent alternative to LLE. SPE originated during the late 1970s and early 1980s as a means to preconcentrate aqueous samples that might contain dissolved semivolatile analytes that are amenable to analysis by gas chromatographic determinative techniques. SPE was first applied to sample preparation problems involving clinical or pharmaceutical types of samples and only much later evolved as a viable sample preparation method in TEQA.

The concept of column liquid chromatography as a means to perform environmental sample preparation was not immediately evident after the development of high-performance (once called high-pressure) liquid chromatography (HPLC) using reversed-phase silica as the stationary phase in the late 1960s. This technique is termed reversed-phase HPLC (RP-HPLC). It is also referred to as bonded-phase HPLC and it is estimated that over 75% of HPLC being done today is RP-HPLC. As early as the 1930s, silica, alumina, Florisil, and Kieselguhr or diatomaceous earth were used as solid sorbents primarily for cleanup of nonpolar extractants and the mechanism of retention was based on adsorption. The early realization that hydrophobic surfaces could isolate polar and nonpolar analytes from environmental aqueous samples such as groundwater came about with the successful use of XAD resins whereby as much as 10 L of sample could be passed through the resin (51). By this time, bonded silicas that contained a hydrocarbon moiety covalently bonded were increasingly predominant in HPLC. One way that this rise in prominence for RP-HPLC can be seen is by perusing the earlier and pioneering text on HPLC (52) and comparing the content in this text to a recently published text by the same authors (53).

It becomes clear that during this 20+-year period, RP-HPLC dominated the practice of column liquid chromatography. It was also becoming evident that the demands being made by EPA and state regulatory agencies

on environmental testing labs required that sample extracts be made as free of background interferences as possible. Terms began to be used such as “the quality of the chromatogram is only as good as the extract.” A well-used cliché also applied “garbage into the GC–MS, garbage out.” All EPA methods during the 1980s that considered aqueous samples required LLE as the sole means to prepare environmental samples for TEQA. LLE was done on a relatively large scale whereby 1000 mL of groundwater or other environmental sample was extracted three times using 60 mL of extracting solvent each time. Because only 1 μ L of extract was required, this left almost all of the extractant unused. As MDLs began to go to lower and lower values due in large part to regulatory pressures, it became evident that the 120 mL of extract could be reduced to 5 mL or less via some sort of vaporization of the solvent. This led to the use of lower-boiling solvents such as methylene chloride (dichloromethane), whose boiling point is 34°C. After all of this, the reduced volume of extract still had to be cleaned up so as to obtain a good signal-to-noise ratio and, hence, to satisfy the now stringent MDL requirements.

The evolution of RP-SPE came about after the development of RP-HPLC. The concept that the hydrophilic silica gel, as a stationary phase, that had been used to pass a nonpolar mobile phase across it could be transformed to a hydrophobic stationary phase was a significant development. Organic compounds whose polarity ranges from *moderate* to *nonpolar* could be retained on these hydrophobic sorbents provided that the mobile phase was significantly more polar than that of the stationary phase. If the particle size could be decreased down to the smallest mesh size, a sorbent material with a relatively large surface area would provide plenty of “active sites.” A surface that was also significantly hydrophobic as well would facilitate removal of moderately polar to nonpolar analytes from water. The stage was set then to bring RP-SPE into the domain of TEQA! This was accomplished throughout the 1980s and led to a plethora of new methods, applications, and SPE suppliers. EPA, however, could not at first envision a role for SPE within its arsenal of methods and hence largely ignored these developments. It did, however, offer to fund some researchers who were interested in demonstrating the feasibility of applying SPE to environmental samples. It became necessary then for analytical chemists engaged in method development to conduct fundamental studies to determine the percent recovery of numerous priority pollutant semivolatile organics from simulated or real environmental samples. This was all in an attempt to validate methods that would incorporate the SPE technique. This author was one such researcher who, while employed in a contract lab in the late 1980s, received a Phase I Small Business Innovation Research (SBIR) grant to conduct just such studies (54).

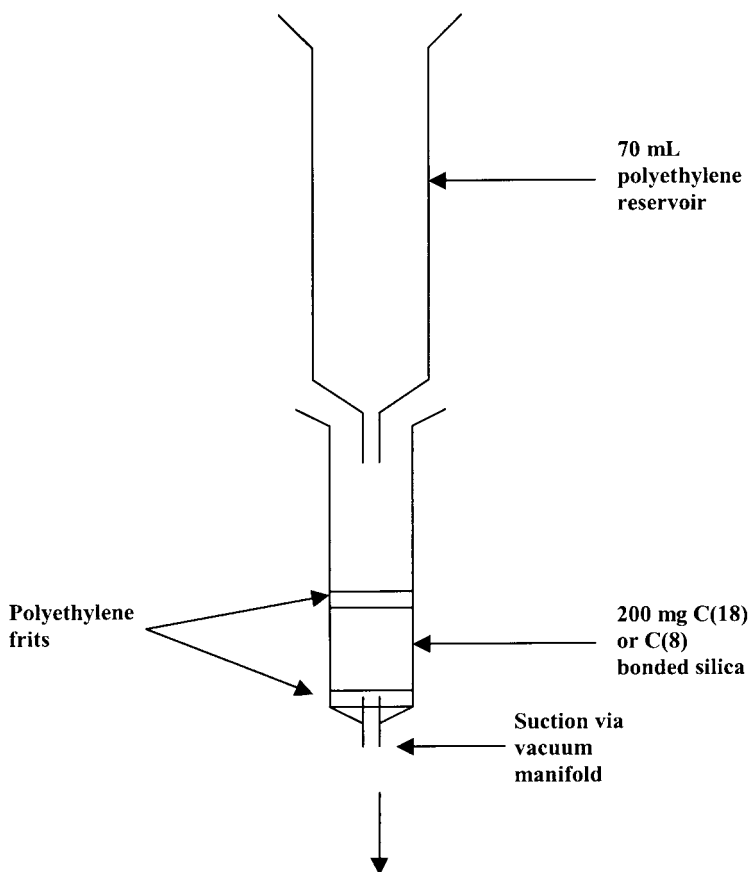
As is true for any emerging and evolving technology, SPE began with a single commercially available product, the Sep-Pak cartridge, developed in 1978 at Waters Associates. These devices, designed to fit to a standard liquid-handling syringe, were at first marketed to the pharmaceutical industry. The focus was on a silica cartridge for what would be considered today as the practice of normal-phase SPE (NP-SPE). The idea that a large volume of sample that could exceed 10 mL led to the development in 1979 to the so-called “barrel” design. Analytichem International, now Varian Sample Preparation Products, was first to introduce the SPE barrel, which, in turn, could be fitted to a vacuum manifold. This design, shown schematically on the next page, consisted of a cylindrical geometry that enabled a larger reservoir to be fitted on top while the Luer tip on the bottom of the barrel was tightly fitted to the inlet port of a vacuum manifold. The vacuum manifold resembled the three-dimensional rectangular-shaped thin-layer chromatographic development tank! The sorbent bed consisted of chemically bonded silica of irregular particle size with a 40- μm average and was packed between a top and a bottom 20- μm polypropylene frit. This configuration “opened the door” to the application of SPE techniques to environmental aqueous samples such as drinking water. It was left to researchers to demonstrate that priority pollutant organics dissolved in drinking water could be isolated and recovered at to at least the same degree as was well-established using LLE techniques. J.T. Baker followed and began to manufacture SPE cartridges and syringe formats in 1982. They are credited with coining the term “SPE” as opposed to the EPA’s term “liquid–solid extraction.” Further evolution of the form that SPE would take was sure to follow.

In 1989, the bulk sorbent gave way to a disk format in which 8–12 μm C_{18} silica was impregnated within an inert matrix of polytetrafluoroethylene (PTFE) fibrils in an attempt to significantly increase the volumetric flow rate of water sample that could be passed through the disk. These developments were made by the 3M Corporation. They coined the term “Empore Disk.” The next major development in SPE design occurred in 1992 as Supelco introduced a device called solid-phase microextraction (SPME). This followed the pioneer developments by Pawliszyn and co-workers (55). The GC capillary column, a topic to be discussed in Chapter 4, was simply “inverted.” The polydimethylsiloxane polymer coating was deposited on the outer surface of a fused-silica fiber. This fiber is attached to a movable stainless-steel “needle in a barrel” syringe. This design is similar to the 7000 series manufactured by the Hamilton Company for liquid-handling microsyringes. SPME is a solventless variation of SPE in that the coated fiber is immersed within an aqueous phase or in the headspace above the aqueous phase. After a finite period of time, the fiber is removed from the sample and immediately inserted directly into the hot-injection port of a GC. The ana-

lytes are then removed from the fiber by thermal desorption directly into a GC column. We will discuss the principles and practice of SPE and then focus on RP-SPE as applied to TEQA. Some of the work of the author will also be included.

48. HOW IS SPE DONE?

A schematic of the basic setup for stand-alone SPE using a large sample volume follows:



The 70-mL reservoir is joined to the SPE cartridge via a plastic adapter. The reservoir and packed cartridge are connected to a vacuum manifold. A reduced pressure is necessary to pass a water sample through the 40- μ m-particle-size sorbent.

All forms of SPE generally follow the same four-step procedure. This process comprises: (1) sorbent conditioning, (2) passage of the sample through the sorbent, commonly called sample loading, (3) removal of interferences, and (4) elution of the analyte of interest into a receiving vessel. The four steps that are involved in performing methods that require SPE as the principal means of sample preparation are now discussed:

1. **Sorbent conditioning:** This initial step is particularly important in order to maximize the percent recovery when using RP-SPE with an aqueous sample. Also, once conditioned, the sorbent should not be allowed to dry. If dried, the sorbent should be reconditioned. For RP-SPE, methanol (MeOH) is usually used to condition, whereas for NP-SPE, *n*-hexane is common. The need for conditioning in RP-SPE has been discussed in the literature (56). The brushlike nature of the organo-bonded hydrophobic surface of the bonded silicas tend to interact and thus render the surface somewhat impermeable. It is as if a thin hydrophobic membrane has been placed on top of the surface. Organics dissolved in water upon passing through this sorbent only partially penetrate this semipermeable membrane. If the sorbent is not conditioned, low and variable percent recoveries from RP-SPE will result. Upon passing MeOH or other polar solvent across the sorbent, the brushes "line up" and, by interacting with MeOH, become more "wetted." This hydrophobic "membrane" becomes permeable and hence dissolved organics can more effectively penetrate this hypothetical membrane barrier. Conditioning has been thought of in terms of a "brush-like." This author has found that by careful adjustment of the level of MeOH by removing trapped air between the reservoir and cartridge in the barrel-type SPE column, MeOH can be passed through the sorbent, followed by the water sample, without exposing the sorbent to air. Some analysts, after conditioning, will pass the eluting solvent through the wetted sorbent. The eluting solvent in many cases of RP-SPE is much less polar than MeOH. In this way, the sorbent can be cleaned of organic impurities. This is also a useful technique to recondition and reuse SPE cartridges. This author has reported even slightly higher % recoveries when conducting RP-SPE on a previously used cartridge!

2. **Sample Loading:** The second step in SPE involves the passage of the sample through the previously conditioned sorbent. The sorbent might consist of between 100 mg and 1 g of bonded silica. The two most common hydrophobic bonded silicas are those that contain either a C₈ or a C₁₈ hydrocarbon moiety chemically bonded to 40- μ m-particle-size silica gel. Alternatively, a disk consisting of impregnated C₈ or C₁₈ silica in either a teflon or glass-fiber matrix is used. For RP-SPE, an aqueous water sample is usually passed through the sorbent. Samples that contain particulates or suspended solids are more difficult to pass through the sorbent. Eventually, the top retaining frits will plug. This drastically slows the flow rate, and if the top frit gets completely plugged, there is no more SPE to be done on that particular cartridge! Suspended solids in water samples present a severe limitation to sample loading. A filtration of the sample prior to SPE sample loading will usually correct this problem. Adding the 70-mL reservoir on top of the SPE barrel-type cartridge enables a relatively large sample volume to be loaded. Upon frequent refilling of the reservoir, a groundwater sample of volume greater than the 70-mL capacity up to perhaps 1000 mL is feasible. Alternatively, the top of the 70-mL reservoir can be fitted with an adapter and a plastic tube can be connected to a large beaker containing the groundwater sample. The design of the multiport SPE vacuum manifold enables 5 or 10 or more samples, depending on the number of ports, to be simultaneously loaded.
3. **Removal of Interferences:** The third step in SPE involves passing a wash solution through the sorbent. The analytes of interest have been retained on the sorbent and should not be removed in this step. In RP-SPE, this wash solution is commonly found to be distilled deionized water. In other cases, particularly when ionizable analytes are of interest, water that has been buffered so that the pH is fixed and known is used as the wash solution. The wash solution should have a solvent strength not much different from that of the sample so that retained analytes are not prematurely removed from the sorbent. In addition, air can be passed through the sorbent to facilitate moisture removal. It is common to find droplets of water clinging to the inner wall of the barrel-type cartridge. Passing air through during this step is beneficial. Also, a Kim-Wipe or other clean tissue can be used to remove surface moisture prior to the elution step. Removal of surface water droplets requires disassembly of the reservoir–adapter–barrel. If a full set of SPE cartridges is used, it is a bit time-consuming to

complete. At this point in the process, the sorbent could be stored or transported. Too few studies have been done to verify or to refute the issue of whether or not analytes are stable enough to be sampled in the field and then transported to the laboratory.

4. **Elution of Retained Analyte:** The fourth and last step in SPE involves the actual removal of the analyte of interest; hopefully, the analyte is free of interferences. This is accomplished by passing a relatively small volume of a solvent, called an eluent, whose solvent strength is very strong so that the analyte is removed with as small a volume of eluent as is possible. This author has been quite successful, particularly when using barrel-type SPE cartridges, in using less than 1 mL of eluent in most cases. A common practice is to make two or three successive elutions of sorbent such that the first elution removes >90% of the retained analyte and the second or third removes the remaining 10%. If there is evidence of water in the receiving vessel, the analyst can add anhydrous sodium sulfate to the receiving vessel. This is particularly relevant for RP-SPE after passage of a drinking water or groundwater sample.

These four steps, which comprise the contemporary practice of SPE, serve to transform an environmental sample that is, by itself, incompatible with the necessary determinative technique, most commonly being gas chromatography. Figure 3.18 is a flowchart to assist the environmental analytical chemist in deciding how to approach analytical method development utilizing SPE. The logic used strongly depends on the chemical nature of the semivolatile analyte of interest and on the sample matrix in which the analyte of interest is dissolved. SPE is applicable only to semivolatile to non-volatile organic analytes. The use of a vacuum to drive the aqueous sample through the sorbent eliminates any applicability that SPE might have in isolating volatile organics.

49. HOW CAN I LEARN MORE ABOUT SPE TECHNIQUES?

The analytical chemistry literature contains novel research that utilizes SPE as the principal sample preparation technique. Major journals in which it is likely that research using SPE will be presented include *Analytical Chemistry* and the applied biennial reviews, *Journal of Chromatography*, *Journal of Chromatographic Science*, *Analytica Chimica Acta*, *LC-GC: The Magazine of Separation Science*, *Environmental Testing & Analysis*, *American Laboratory*, *American Environmental Laboratory*, and *Environmental Science and Technology*.

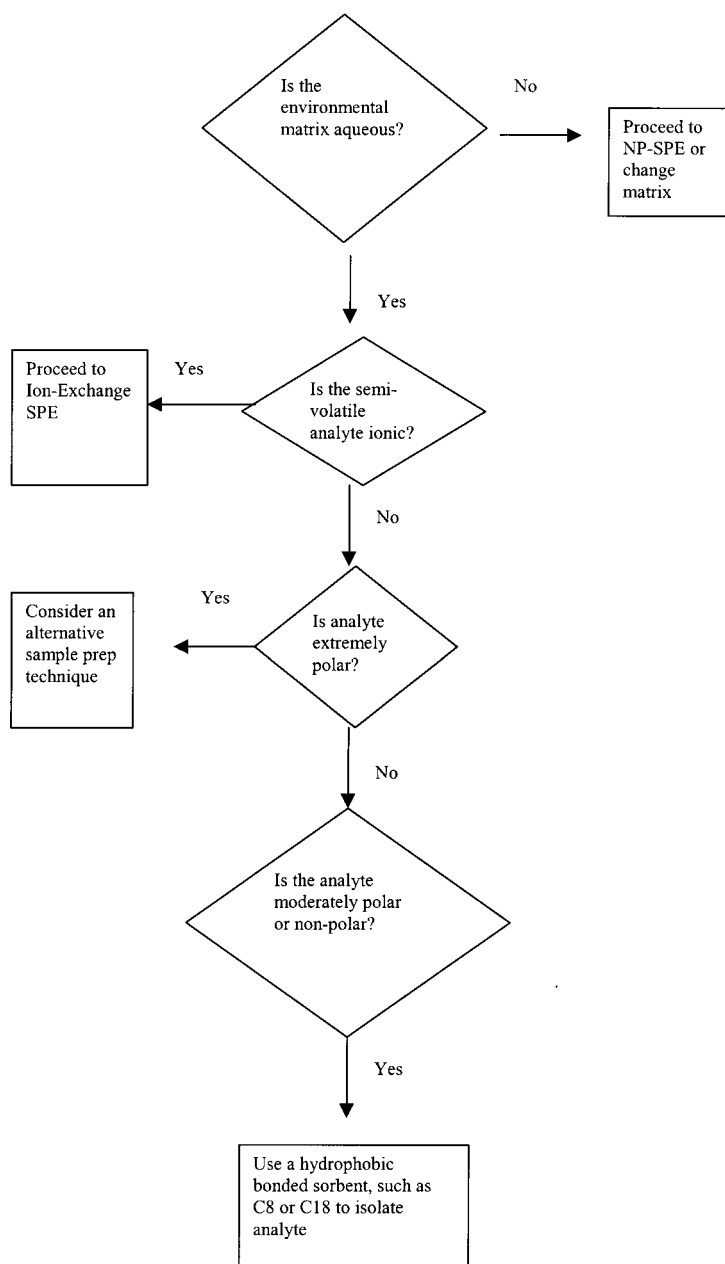


FIGURE 3.18 Flowchart for analytical method development using SPE techniques.

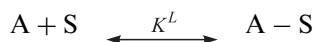
A chapter in a popular book on HPLC nicely presents SPE (57). Two texts have recently been published on the principles and practice of SPE (58). Recently, a special issue of *LC-GC: The Magazine of Separation Science* was devoted to sample preparation and included articles that addressed not only conventional SPE but included automated SPE, matrix solid-phase dispersion, membrane filtration, solid-phase microextraction, and polymeric RP-SPE sorbents (59). *Cahners Business Information*, that publishes *R & D Magazine* has recently started a monthly newsletter titled "Sample Preparation" largely as a means to showcase products related to SPE and related techniques.

50. HOW DOES SPE WORK?

Solid-phase extraction works, that is, the bonded silica removes moderately polar to nonpolar solutes from an aqueous matrix based on thermodynamic principles. These principles were introduced early in this chapter as a means to discuss the underlying physical chemistry of LLE. Equation (3.4) can be restated pertinent to SPE as follows:

$$K^o = \frac{a_{\text{surface}}}{a_{\text{aq}}}$$

This relationship resulted from the spontaneous tendency for the analyte, initially solubilized in an aqueous matrix, to partition into the surface monolayer of hydrophobic octadecyl- or octyl-bonded silica. Organic compounds as analytes will have a unique value for the thermodynamic distribution constant. One fundamental difference between LLE and SPE stands out! Partition or adsorption of solute molecules onto a solid surface follows the principles of the Langmuir adsorption isotherm, whereas LLE does not. We will briefly develop the principles below. This model assumes that an analyte A combines with a site of adsorption, S, in which there is a finite number of such sites according to



An equilibrium constant, K^L , can be defined in terms of the mole fraction of adsorbed sites, $X_{\text{A-S}}$, the mole fraction of unadsorbed sites, X_{S} and the mole fraction of analyte in equilibrium according to, X_{A} :

$$K^L = \frac{X_{\text{A-S}}}{X_{\text{S}}X_{\text{A}}}$$

A fractional coverage, θ , can be defined whereby

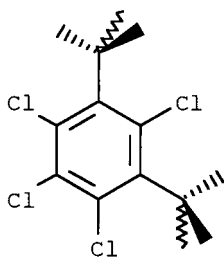
$$\theta = \frac{K^L X_A}{1 + K^L X_A}$$

When the mole fraction of analyte is low, the above relationship can be simplified to

$$\theta = K^L X_A$$

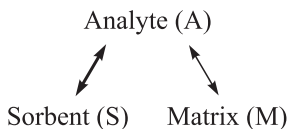
When the mole fraction of analyte is high, θ approaches 1. These equations can be visualized graphically in Figure 3.19.

An organic compound such as tetrachloro-*m*-xylene (TCMX, whose structure is shown below) and initially dissolved in water, is thermodynamically unstable.



Tetrachloro-*m*-xylene

Upon passage of a water sample containing TCMX through a chemically bonded silica sorbent, the degree of interaction between the sorbent surface and TCMX exceeds the degree of interaction between the aqueous matrix and TCMX. In other words, the free energy of interaction between the sorbent and TCMX, the analyte-sorbent interaction, is lower than that for the analyte-matrix interaction. In other words, the minimum in the free energy curve, as shown in Figure 3.2, occurs well displaced toward the side of the analyte-sorbent interaction. This concept can be viewed as an “eternal triangle” whereby both the sorbent and matrix compete for the analyte:



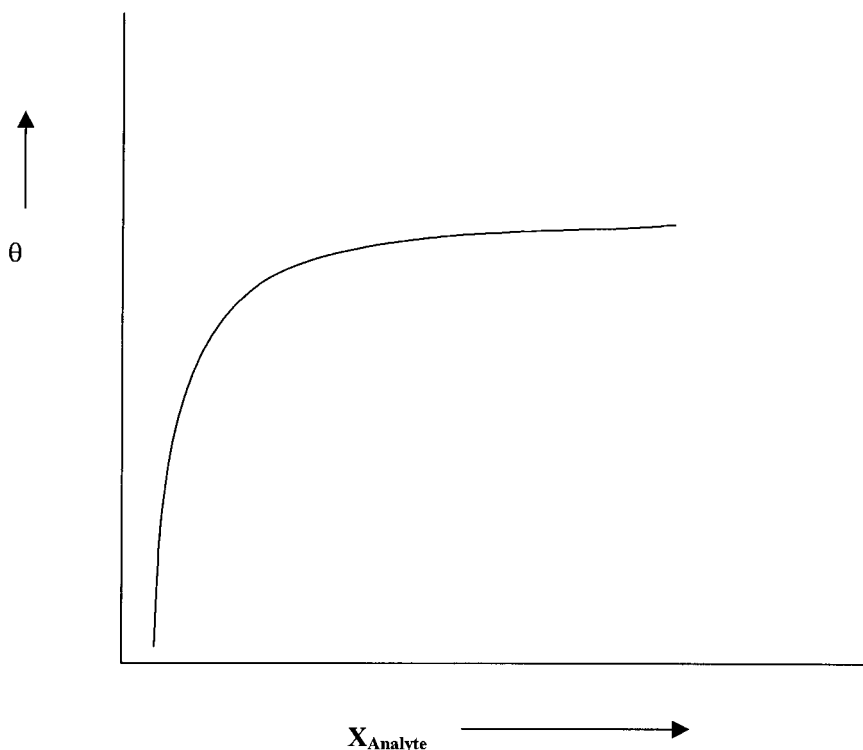


FIGURE 3.19 Plot of the fractional coverage versus the mole fraction of analyte that obey the Langmuir adsorption isotherm.

Efficient removal of analyte from the matrix requires that the strength of the A–S interaction be increased while that of the A–M interaction be decreased. This is accomplished chemically by matching the polarity of the sorbent to that of the analyte while “mismatching” the polarity of the matrix to that of the analyte. Consider TCMX as the analyte and octyl-bonded silica as the sorbent. If TCMX could be dissolved in water (a very small amount of TCMX can actually dissolve in water until a saturated solution is reached), an aqueous sample would be created, and if ultrapure water was used, this sample would be known as a “spiked” sample. One way to increase the amount of TCMX that can be dissolved in a given volume of water is to spike the water with a methanolic solution. Methanol serves as a molecular interface by enabling TCMX to permeate the hydrogen-bonded matrix. The solubility of TCMX is now significantly increased and a series of spiked samples can be prepared that cover a wide range of solute concentration.

51. IS SPE A FORM OF COLUMN CHROMATOGRAPHY?

Yes, SPE, as a form of liquid column chromatography, is one of three broad classifications of column chromatography. Historically, SPE would have been called frontal development. The other two categories are elution and displacement chromatography. Elution chromatography is the principal means to conduct GC and HPLC and is of the utmost importance to TEQA and as an instrumental technique; it will be discussed in sufficient detail in Chapter 4. In elution chromatography, a mobile phase is continuously passed across a stationary phase and a small “plug” of sample is “injected” into this flowing stream. Displacement chromatography is similar to elution, except that the mobile phase is much more strongly retained by the stationary phase than is the sample (60). Displacement chromatography is a rarely used form and will not be considered here.

The principles underlying SPE as an example of frontal chromatography can be more easily understood if the following experiment is designed, the instrument configured, and the results interpreted (61). Figure 3.20 is a schematic of a frontal chromatographic instrumental configuration in which the bonded sorbent is placed into a HPLC column and installed in such a way that the sample is passed through the SPE sorbent and the effluent is also passed through a ultraviolet absorbance (UV) detector via a flow-through micro-cell. Provided that the total extra column void volume is not large in comparison to the size of the SPE column, a frontal chromatogram can be obtained. The reservoir contains the analyte dissolved in a matrix such as groundwater, where the analyte–matrix interaction exists (A–M) and the analyte sorbent interaction (A–S) takes over in the SPE column. Figure 3.21 is the frontal chromatogram that would be typical. This assumes that the analyte is a strong absorber in the ultraviolet region of the electromagnetic spectrum. Referring to Figure 3.21, it should be

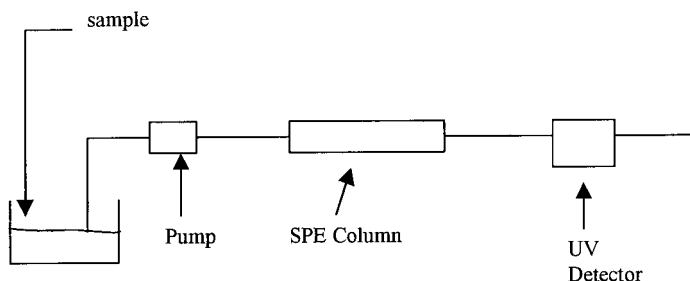


FIGURE 3.20 Schematic of a frontal chromatographic instrumental configuration.

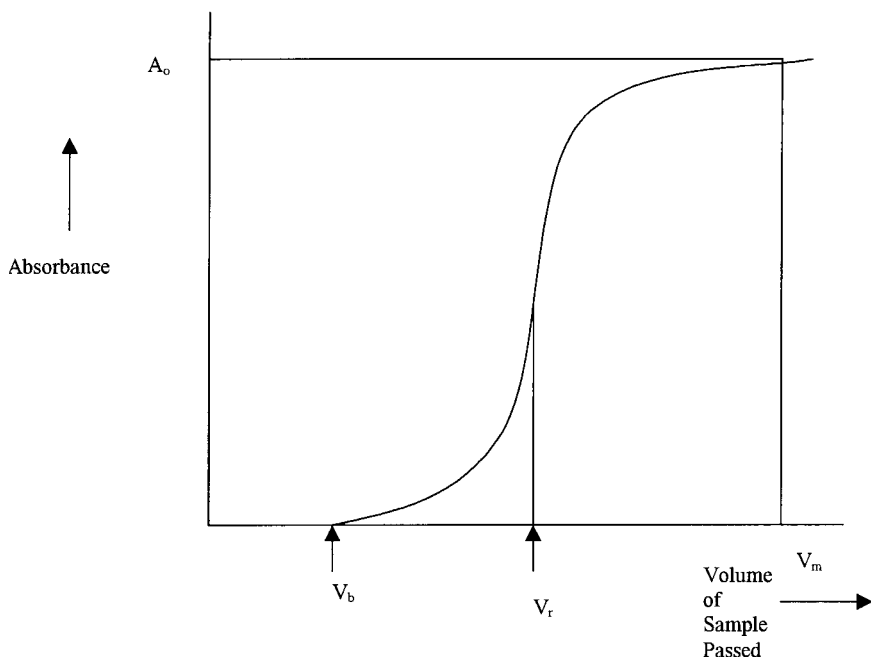


FIGURE 3.21 Plot of absorbance versus the volume of sample passed in SPE breakthrough studies.

noted that there is a certain volume of sample that can be passed through the sorbent without observing any rise in the absorbance. At V_b , a volume that represents the volume of sample that gives an absorbance that is 1% of A_o , the first signs of “breakthrough” are seen to emerge. The inflection point in the curve, denoted by a volume of sample passed through the column, V_r , is unique to a given solute. The absorbance is seen to increase sigmoidally as shown in Figure 3.21 and plateaus at A_o . This plateau, in essence, is the same one as shown by the Langmuir isotherm in Figure 3.19.

Let us develop this concept a bit more by assuming that approximately 200 mL of water has been spiked with traces of dimethylphthalate (DMP). DMP strongly absorbs in the UV and one can expect a sigmodal curve as shown in Figure 3.21 to result. If, instead of DMP, dibutylphthalate (DBP) is added to water, a similar curve is obtained. In this case, a significant increase in V_b and V_r is observed. This is shown in Figure 3.22. If both DMP and DBP were spiked together at the onset to 200 mL of high-purity water and the breakthrough experiment performed, the curve would resemble that shown in Figure 3.23. Much before the commercial development of

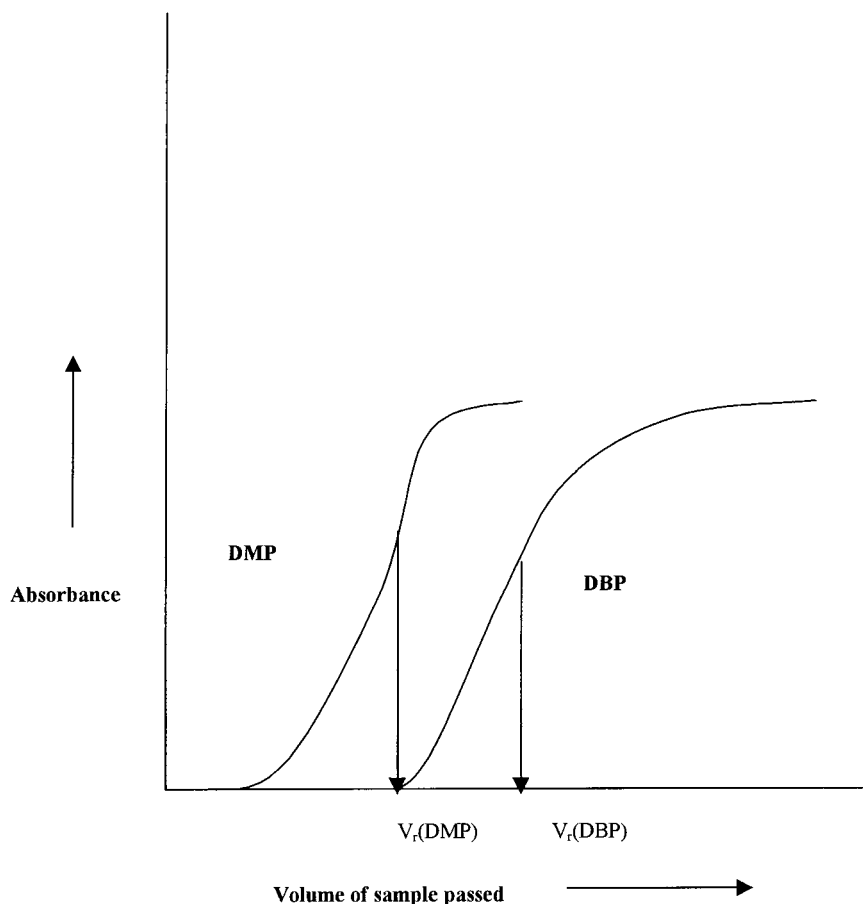


FIGURE 3.22 Plot of absorbance versus the volume of sample passed for two analytes injected independently, DMP and DBP.

SPE, a curve such as that shown in Figure 3.23 was already established and the following predictions made (60):

separation results in the formation of fronts ... the least retained component emerges first from the bed followed by a mixture of the least and next most strongly retained component. ... This technique is useful for concentrating trace impurities ...

This author doubts whether these authors could have foreseen the impact of their words on the eventual development of commercial products that rely on frontal development, namely SPE.

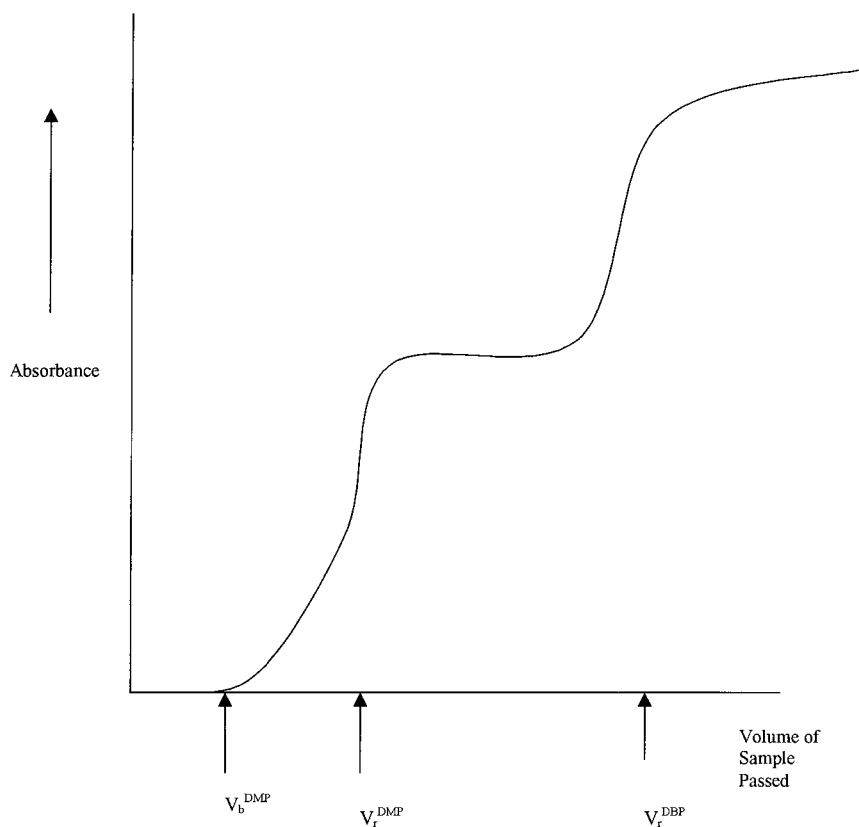


FIGURE 3.23 Plot of absorbance versus the volume of sample passed for a mixture of DMP and DBP.

Let us now set aside this spiked aqueous sample that consists of DMP and DBP dissolved in water. This sample easily simulates an environmental sample that contains phthalate esters since this class of organic compound is commonly found in landfill leachates due to the use of phthalate esters as plasticizers. Let us reconfigure our HPLC to that shown in Figure 3.24, so that ultrapure water is being pumped through our SPE column. The results, shown in Figure 3.25, corresponding to elution chromatography, give instead of the sigmoidal shape, the Gaussian peak profile with the apex of the peak located at V_r . Because V_r differs between DMP and DBP, we have a basis of separation. We will discuss the theory underlying column elution liquid chromatography as a means to separate and to quantitative the presence of organic compounds in Chapter 4.

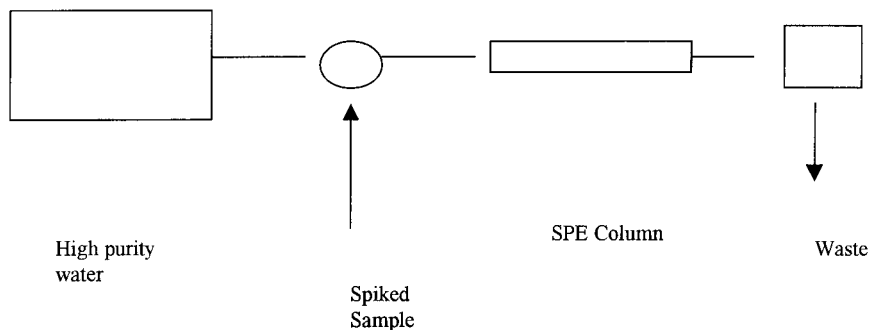
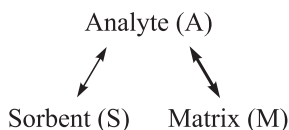


FIGURE 3.24 Schematic of an elution chromatographic instrumental configuration.

Solid-phase extraction has been called digital chromatography because its chief use is to retain and then remove analytes of interest in an “on–off” fashion. Outside of conducting breakthrough studies, the purely frontal chromatographic form has little practical value in TEQA. The elution step, in which an eluent whose polarity is near to that of the analyte, removes all analytes and can be viewed in a manner similar to that done for the sorption step:



The analyte–matrix interaction becomes so strong that analytes are easily and, therefore, quickly eluted from the sorbent. The polarity of the matrix now matches the polarity of the analyte.

52. CAN SPE BREAKTHROUGH BE PREDICTED?

We saw earlier just how V_b and V_r are obtained experimentally by reconfiguring a high pressure liquid chromatograph (refer to Figure 3.20). A HPLC column packed with SPE sorbent replaces the conventional HPLC column. A spiked sample is pumped through the SPE column to develop the frontal chromatograms as shown in Figure 3.21. This configuration is all it takes to conduct the breakthrough study. Is there sufficient theory developed to predict V_b and/or V_r ? Yes there is and we proceed to con-

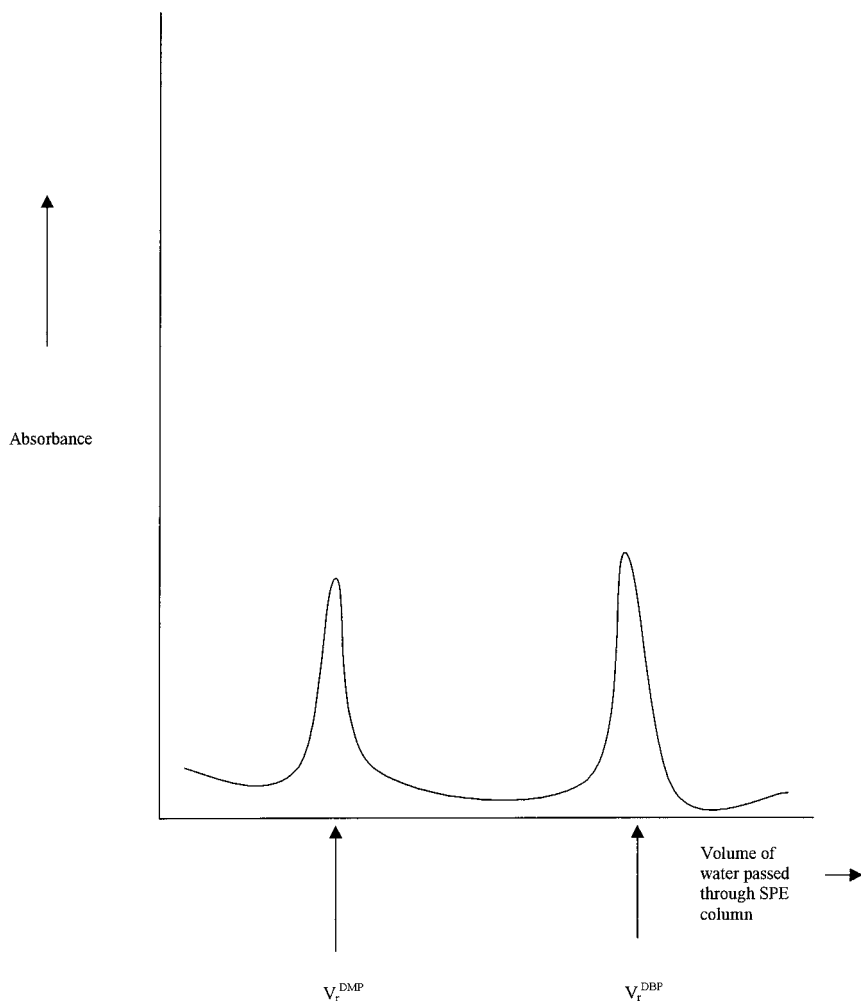


FIGURE 3.25 Chromatogram from the elution chromatography instrumental configuration.

sider this below. Before we do this, we need to introduce certain concepts and definitions that are fundamental to achieving a somewhat deeper understanding of liquid chromatography. We must introduce the important elements from the more contemporary approaches taken to understand exactly what one means by the term *solvent polarity*. We must also discuss what one means by the term *aqueous solubility* for an organic

compound acting solute and we need to discuss how this important parameter relates to the solute's octanol–water partition coefficient. We admit to digressing somewhat into the physical chemistry of solution behavior insofar as it aids the reader in understanding the theory that underpins RP-SPE. Introducing these concepts in this chapter on sample preparation will facilitate the discussion of HPLC as a determinative technique in the next chapter.

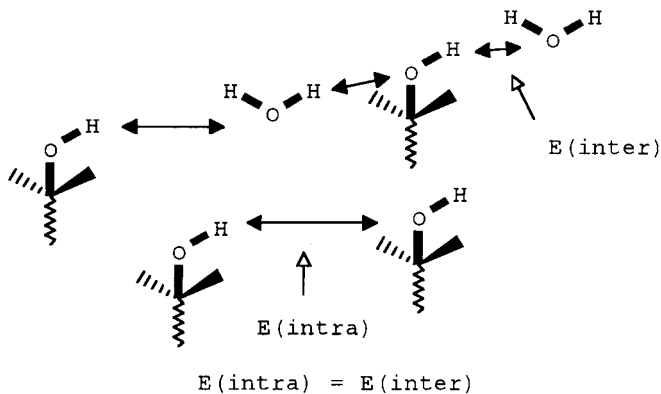
53. WHAT IS SOLVENT POLARITY?

Solvent polarity is a numerical value that can be assigned to each of the common organic solvents used in TEQA. A solvent polarity index, denoted by P' , is defined based on experimental solubility data using test solutes and was originally developed by Rohrschneider (62) and further discussed by Snyder (63,64). Table 3.9 lists a number of representative solvents in order of increasing P' (65). The introduction of heteroatoms such as oxygen, sulfur, and nitrogen significantly contribute to solvent polarity. Consider what happens when a test solute such as methanol is added to the very polar solvent water versus what happens when methanol is added to the very nonpolar solvent hexane. The classic cliché, “like dissolves like” certainly applies whereby methanol whose P' is 5.1 is infinitely soluble or completely miscible in all proportions with water whose P' is 10.2. However, methanol, with a density of 0.7915 g/mL at 20°C, will tend to sink to the bottom when added to hexane, whose density is 0.660 g/mL at the same temperature. The presence of hydroxyl groups in both methanol and in water creates an infinite number of hydro-

TABLE 3.9 Polarity of Selected Organic Solvents

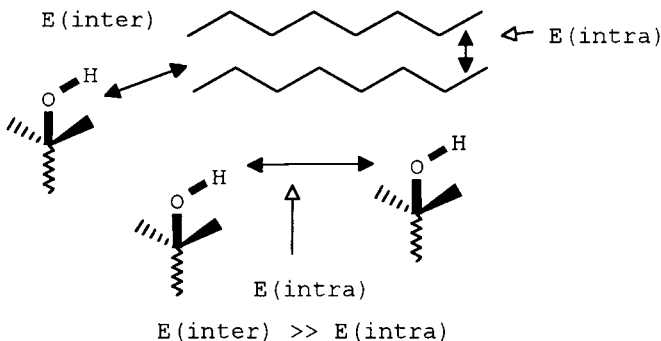
Solvent	P'
Hexane	0.1
Cyclohexane	0.2
Toluene	2.4
Ethyl ether	2.8
<i>n</i> -Propyl alcohol	4.0
Methylethyl ketone	4.7
Methanol	5.1
<i>N,N</i> -dimethyl formamide	6.4
Dimethylsulfoxide	7.2
Water	10.2

gen bonds and is responsible for defining both solvents as associated liquids. The degree to which methanol molecules interact through intramolecular hydrogen bonding is of a similar attractive potential energy, as is the degree to which water molecules interact through intramolecular hydrogen-bonding. The energy of interaction is due to hydrogen-bonding between methanol and water, the so-called intermolecular hydrogen-bonding; this is shown in the following:



Because there is little difference in the potential energy for the interaction intramolecularly and intermolecularly, the tendency for matter to spread (entropy) becomes the driving force between methanol and water.

Consider what happens when the two immiscible liquids, methanol whose P' is 5.1 and hexane whose P' is 0.1, are in contact with each other. The weak dispersion forces are present throughout hexane, whereas the much stronger interaction due to hydrogen-bonding among methanol molecules exist; this is shown in the following:



It is impossible to overcome the high intermolecular potential energy that exists to afford a true dissolution of hexane into methanol and vice versa. These intramolecular and intermolecular forces that exist among molecules in liquids as depicted above not only serve to explain why hexane and methanol do not “mix” and remain as two immiscible liquids but also help in understanding how to go about developing a method that incorporates SPE as the principal sample preparation technique. Hexane is a suitable elution solvent for relatively nonpolar analytes that are sorbed onto and into the reversed-phase material, whereas methanol might not be as suitable in terms of efficient elution from the same SPE sorbent.

54. CAN A SOLUTE'S AQUEOUS SOLUBILITY OR OCTANOL-WATER PARTITION COEFFICIENT BE USED TO PREDICT RP-SPE % RECOVERIES?

Yes, both solute physico-chemical properties give important clues about the possibility of whether or not a given organic compound originally dissolved in an aqueous matrix will be effectively recovered by RP-SPE. Again, because SPE is a two-step transfer of solute (sorption from the matrix followed by elution from the sorbent), both factors serve to limit the % recovery. It is only % recoveries that can be measured by determinative techniques. There is no direct way to measure the amount of analyte sorbed except through the breakthrough experiments previously discussed. Let us assume then that the analyte, once sorbed, is efficiently removed from the sorbent. The differences in our measured % recoveries then reflect only the mass transfer of analyte from an aqueous matrix to the chemically bonded sorbent. Solutes that in general exhibit low aqueous solubilities, S_{aq} , and have relatively large octanol–water partition coefficients, K_{ow} , have a strong tendency to sorb on hydrophobic surfaces from an aqueous matrix. The mathematical relationship between S_{aq} and K_{ow} has been the focus of extensive study among physical chemists. A nearly inverse and linear relationship exists between the two solute parameters. This is stated from one resource as follows (66):

$$\log K_{ow} = -\log S_{aq} - \log \gamma_0 - \log V_0 \quad (3.36)$$

where γ_0 represents the activity coefficient for a water saturated octanol phase and V_0 is the molar volume for the water saturated octanol phase, which is 0.12 L/mol. If $\log K_{ow}$ is plotted against the values for the aqueous solubility, S_{aq} , for a variety of organic compounds of environmental interest, a linear relationship with a negative slope emerges. The majority of

priority pollutant organic compounds have activity coefficients in water-saturated octanol, γ_0 , that fall between values of 1 and 10. This suggests that these solutes exhibit ideal solution behavior and reflects a strongly hydrophobic character (66).

In general, polar solutes have $\log K_{ow}$ values less than 1; moderately polar solutes have $\log K_{ow}$ values between 1 and 3; nonpolar solutes have $\log K_{ow}$ greater than 3. To illustrate the influence of various substituents on the $\log K_{ow}$ for a parent compound such as phenol, consider the following list of phenols (67):

Phenol	$\log K_{ow}$
Phenol	1.46
<i>o</i> -Methylphenol	1.95
<i>o</i> -Chlorophenol	2.15
2,6-Dimethylphenol	2.36
<i>p</i> -Chlorophenol	2.39
2,4-Dichlorophenol	3.08
<i>p</i> -trimethylsilylphenol	3.84

Clearly, the effect of either chloro or methyl substitution serves to significantly increase the octanol–water partition coefficient. This increase in hydrophobicity is an important consideration in developing a RP-SPE method that is designed to isolate one or more phenols from a ground-water sample. Those substituted phenols with the greater values for K_{ow} would be expected to yield the higher % recoveries in RP-SPE. The substitution of a trimethylsilyl moiety in place of hydrogen significantly increases the hydrophobicity of the derivatized molecule. This use of a chemical derivative to convert a polar molecule to a relatively nonpolar one has important implications for TEQA and will be discussed in Chapter 4.

55. WHAT IS AQUEOUS SOLUBILITY ANYWAY?

The amount of solute that will dissolve in a fixed volume of pure water is defined as that solute's aqueous solubility, S_{aq} . It is a most important concept with respect to TEQA because there is such a wide range of values for S_{aq} among organic compounds. Organic compounds possessing low aqueous solubilities generally have high octanol–water partition coefficients. This leads to a tendency for these compounds to bioaccumulate. The degree to which an organic compound will dissolve in water depends not only on the degree of intramolecular versus intermolecular interactions as we saw earlier but also on the physical state of the substance (i.e., solid, liquid, or

gas). The solubility of gases in water is covered by Henry's Law principles as discussed earlier.

A solute dissolved in a solvent has an activity, a . This activity is defined as the ratio of the solute's fugacity (or tendency to escape) in the dissolved solution to that in its pure state. The activity coefficient for the i th solute, γ_w^i , relates solute activity in pure water to the concentration of the solute in water where the concentration is defined in units of mole fraction, x_w , according to

$$x_w^i = \frac{\text{moles}^i}{\sum_i \text{moles}^i}, \quad \gamma_w^i \equiv \frac{a_w^i}{x_w^i}$$

We usually think of aqueous solubilities in terms of the number of moles of the i th solute per liter of solution. Alternative units commonly used to express aqueous solubility include milligrams per liter or parts per million and also micromoles per liter. The concentration in moles per liter can be found by first considering the following definition:

$$C_i = \frac{x_i(\text{mole/mole total})}{V_{\text{mix}}(\text{L/mole total})}$$

Because V_w , the molar volume of water, is 0.018 L/mol and V_i , the molar volume of a typical solute dissolved, the molar volume of the mixture, V_{mix} , is found by adding the molar volume contributions of the solute and the solvent, water according to

$$V_{\text{mix},w} = 0.2x_{i,w} + 0.018x_w$$

This expression can be further simplified to

$$V_{\text{mix},w} = 0.182x_{i,w} + 0.018$$

Because the mole fraction for solutes dissolved in water is very low, $x_{i,w} < 0.002$, which corresponds to about 0.1M, the molar volume for the mix can be approximated at 0.018 and hence the above equation for the concentration in moles per liter can be expressed as

$$C_w^{\text{sat}} = \frac{x_w^{\text{sat}}}{V_{\text{mix},w}} \cong \frac{x_w^{\text{sat}}}{0.018} (\text{moles/L})$$

The above equation converts the mole fraction of a solute dissolved in water, up to saturation conditions, x_w^{sat} , to the corresponding concentration of that solute in units of moles per liter. By knowing the molecular weight of a particular solute, it is straightforward to obtain the corresponding concentration in milligrams per liter.

For hydrophobic solutes, γ_w is greater than 1. When enough solute has been added to a fixed volume of water until no more can be dissolved, the solution is said to be saturated and a two-phase system results. This is a condition of dynamic equilibrium whereby the solute activity in both phases is equal. Denoting o as the organic phase, we have:

$$a_w = a_o$$

so that

$$x_w \gamma_w = x_o \gamma_o$$

For immiscible liquids in water, the mole fraction of solute in itself and its activity coefficient approximate 1 so that

$$x_w \gamma_w = 1$$

and the mole fraction, x_w , is related to the aqueous activity coefficient by

$$x_w = \frac{1}{\gamma_w}$$

and in terms of logarithms,

$$\log x_w = -\log \gamma_w$$

The purpose of deriving this is to show that attempts to measure the aqueous solubility, x_w , are, in essence, attempts to estimate the activity coefficient, γ_w . This relationship applies only to liquid solutes. Solid or crystalline solutes require an additional term where

$$\log x_w = \log \frac{x^c}{x_{\text{scl}}} - \log \gamma_w$$

where x_c/x_{scl} represents the ratio of the solubility of the crystal to that of the hypothetical supercooled liquid (68). The additional term above is approximated by the term $-0.00989(T_{\text{mp}} - 25)$ for rigid molecules at 25°C and by $-[0.01 + 0.002(n-5)] T_{\text{mp}}$ for long-chain molecules with n monomers per

polymer and T_{mp} is the melting point of the solid. Octanol–water partition coefficients can also be estimated from solute retention times using, for example, reversed-phase HPLC.

Aqueous solubility for various solutes can be estimated by applying any of the techniques in the following four categories (69):

1. Methods based on experimental physico-chemical properties such as partition coefficients, chromatographic retention, boiling point, and molecular volume.
2. Methods based directly on group contributions to measured activity coefficients.
3. Methods based on theoretical calculations from molecular structures, including molecular surface area, molecular connectivity, and parachor.
4. Methods based on combinations of two or more parameters that can be experimentally measured, calculated, or generated empirically. These include the solubility parameter method, the UNIFAC technique (a method based on linear solvation energy relationships and on the use of multivariate statistical methods).

However, for many of the very hydrophobic solutes of interest to TEQA, the aqueous solubility is extremely low. For example, iso-octane, a nonpolar hydrocarbon, will dissolve in water up to 0.0002% at 25°C (70). Higher-order aliphatic hydrocarbons have such low values for their aqueous solubilities as to make it impossible to directly measure this physico-chemical property.

56. WHAT DOES A PLOT OF K_{ow} VERSUS AQUEOUS SOLUBILITY TELL US?

Figure 3.26 is a qualitative plot of the logarithm of the octanol–water partition coefficient, K_{ow} against the logarithm of the aqueous solubility, S_{aq} , of a selected number of organic solutes of environmental interest. This qualitative plot makes it very evident that polar solutes with appreciable aqueous solubility exhibit low values of K_{ow} . Hydrophobic solutes that are not soluble in water to any great extent exhibit large values of K_{ow} . The importance of knowing K_{ow} values as this pertains to the practice of TEQA and to environmental science in general has been stated earlier:

In recent years the octanol/water partition coefficient has become a key parameter in studies of the environmental fate of organic chemicals. It has been found to be related to water solubility, soil/sediment adsorption coefficients, and bioconcentration factors

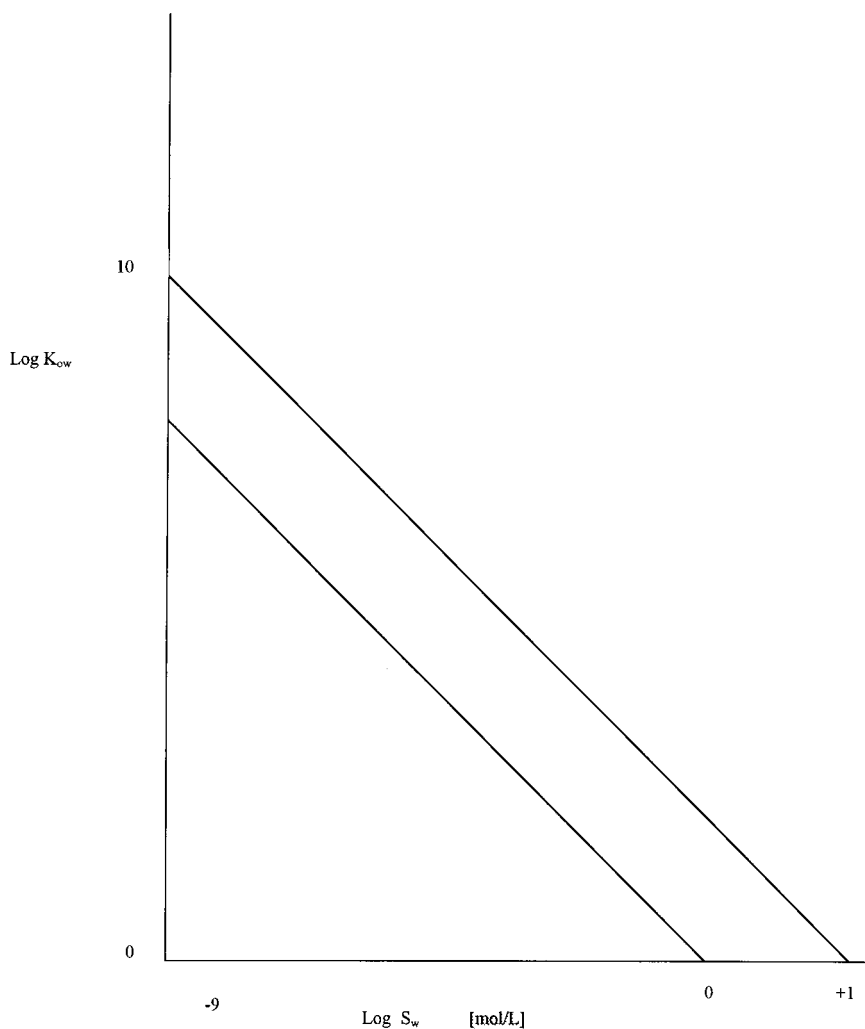


FIGURE 3.26 Plot of the logarithm of the octanol–water partition coefficient versus the logarithm of the aqueous solubility.

for aquatic life. Because of its increasing use in the estimation of these other properties, K_{ow} is considered a required property in studies of new or problematic chemicals. (71)

Much effort has been expended in finding a linear equation that relates octanol–water partition coefficients for any and all solutes to the aqueous

solubility in octanol-saturated water. Two useful equations have emerged in which the logarithm of the aqueous solubility, $\log S_{\text{aq}}$, is, in general, linearly related to $\log K_{\text{ow}}$ (68):

For liquid solutes:

$$\log K_{\text{ow}} = 0.8 - \log S_{\text{aq}} \quad (3.37)$$

For crystalline solutes:

$$\log K_{\text{ow}} = 0.8 - \log S_{\text{aq}} - 0.01(T_{\text{mp}} - 25) \quad (3.38)$$

Yalkowsky and Banerjee have reviewed specific studies and summarized the relationship in Eqs. (3.37) and (3.38) as applied to liquid organic compounds and for crystalline organic solids (68). The results are shown in Table 3.10 for four classes of liquid hydrocarbons. A plot of $\log K_{\text{ow}}$ against $\log S_{\text{aq}}$ reveals a slope close to the value of -1 for each of these four classes of hydrocarbons. The differences in the y intercept reflect differences in the activity coefficients as defined in Eq. (3.36).

57. CAN WE PREDICT VALUES FOR K_{ow} FROM ONLY A KNOWLEDGE OF MOLECULAR STRUCTURE?

Yes, and the predictions are quite good. Two different methods emerge from a host of others and are most commonly used to predict octanol–water partition coefficients for the many organic compounds that exist. One approach is to calculate K_{ow} from a knowledge of structural constants, whereas the second approach requires that a chemical's partition coefficient be measured between a solvent other than octanol and water, K_{sw} . K_{ow} can then be calculated from linear regression equations that relate $\log K_{\text{sw}}$ (for a particular solvent) and $\log K_{\text{ow}}$. Two forms of the structural constant approach are most popular: (1) the Hansch π hydrophobic character of

TABLE 3.10 Degree of Correlation Between the Octanol-Water Partition Coefficient and Aqueous Solubility for Four Classes of Organic Compounds

Class	$\log K_{\text{ow}}$	Corr. coeff
Aromatic hydrocarbons	$0.768-1.056 \log S_{\text{aq}}$	0.995
Unsaturated hydrocarbons	$0.250-0.908 \log S_{\text{aq}}$	0.993
Halogenated hydrocarbons	$0.323-0.907 \log S_{\text{aq}}$	0.993
Normal hydrocarbons	$0.467-0.972 \log S_{\text{aq}}$	0.999

substituents approach and (2) the Leo Fragment constant approach. The Hansch π approach is based on the assignment of a value for π_X as the difference between octanol–water partition coefficients for a substituted versus unsubstituted or parent compound. Mathematically, this difference can be stated as follows:

$$\pi_X = \log K_{ow}^X - \log K_{ow}^H$$

For example, the $\log K_{ow}$ for chlorobenzene is 2.84 whereas that for benzene is 2.13 and thus

$$\pi_X = 2.84 - 2.13 = 0.71$$

and the π_X for the substituent chlorine on the monoaromatic ring is 0.71. The Hansch π approach has been recently criticized because it ignores hydrogens attached to carbon and this led to some erroneous values for a few aliphatic hydrocarbons.

The Leo Fragment method has emerged as a more powerful way to predict $\log K_{ow}$. The working mathematical relationship is

$$\log K_{ow} = \sum_i f_i + \sum_j F_j \quad (3.39)$$

where the logarithm of the octanol–water coefficient for a particular organic compound is found by algebraically summing the contributions due to the i th structural component (f) (building block or functional group) over all components and the algebraic sum of the j th factor (F) due to intramolecular interactions due largely to geometric or electronic effects. For complex molecules, it is desirable to have a known $\log K_{ow}$ value for a given compound and use the fragment approach to add and subtract structural and geometric/electronic contributions such that

$$\begin{aligned} \log K_{ow}(\text{unknown}) = \log K_{ow}(\text{known}) \\ - \sum_{\text{removed}} f + \sum_{\text{added}} f - \sum_{\text{removed}} F + \sum_{\text{added}} F \end{aligned} \quad (3.40)$$

Thus, two ways emerge to calculate $\log K_{ow}$ using the Leo Fragment method. One is to build the entire molecule from fragments and factors using Eq. (3.40) and the other is to calculate $\log K_{ow}$ from structurally related compounds. To illustrate how one goes about calculating the $\log K_{ow}$ for a specific organic compound, we return to TCMX. We begin by first using Eq. (3.39) to establish the $\log K_{ow}$ for benzene by using previously

published fragment factors. For carbon that is aromatic, a fragment factor of 0.13 has been established. For hydrogen that is covalently bonded to aromatic carbon, the fragment factor of 0.23 has been established. Hence, because benzene consists of six aromatic carbons that, in turn, are bonded to six hydrogens,

$$\begin{aligned}\log K_{ow}(\text{C}_6\text{H}_6) &= 6f_{c(\text{aromatic})} + 6f_H^\phi \\ &= 6(0.13) + 6(0.23) \\ &= 2.16\end{aligned}\quad (3.41)$$

This prediction is quite close to the well-established and observed value for benzene, which is 2.13. Using this established value for benzene, we begin to view our molecule of interest, TCMX, as a substituted benzene. Theoretically, we can arrive at TCMX by removing all six aromatic hydrogens and then adding two methyl groups and four chlorine groups. Stated mathematically,

$$\log K_{ow}(\text{TCMX}) = \log K_{ow}(\text{C}_6\text{H}_6) - 6f_H^\phi + 2f_{\text{CH}_3}^\phi + 4f_{\text{Cl}}^\phi \quad (3.42)$$

The methyl group is viewed as being composed of a aromatic carbon and three aliphatic bonded hydrogens and fragment constants and Eq. (3.42) is modified as follows:

$$\log K_{ow}(\text{TCMX}) = \log K_{ow}(\text{C}_6\text{H}_6) - 6f_H^\phi + 2[f_C^\phi + 3f_H] + 4f_{\text{Cl}}^\phi \quad (3.43)$$

Upon substituting the values for the various fragment constants into Eq. (3.43), we get

$$\begin{aligned}\log K_{ow}(\text{TCMX}) &= 2.13 - 6(0.23) + 2[(0.13) + 3(0.23)] + 4(0.19) \\ &= 6.15\end{aligned}$$

Such a large value for the octanol–water partition coefficient for TCMX suggests that the compound exhibits a very low aqueous solubility. Note that the substitution for hydrogen by methyl and chloro groups significantly increases the hydrophobicity of the molecule. The addition of a methyl group is seen to add about 0.5 of a log unit irrespective of the compound involved. For example, substituting a methyl for hydrogen in benzene contributes the same as if a methyl is substituted for hydrogen in cyclohexane.

As long as the building blocks lead to a complete molecular structure, only structural fragment constants are necessary, as in the TCMX example.

However, for molecules that are more complex, the molecular components begin to exert significant influences on one another through steric, electronic, and resonance *intramolecular* interactions. These interactions affect both the aqueous and octanol activity coefficients, as discussed earlier. Geometric effects include double and triple bonds, the ability of groups to rotate about a carbon-carbon single bond, chair/boat conformations in cyclohexane, and carbon-chain branching in aliphatic structures. Geometric effects increase aqueous solubility, decrease K_{ow} , and, hence, their F factor contributes negativity to Eq. (3.40). Electronic effects due to electronegativity differences between the elements of both atoms that comprise a polar covalent bond such as a carbon-to-chlorine bond decrease aqueous solubility, increasing K_{ow} , and, hence, their F factor contributes positively to Eq. (3.40). Other electronic effects include nearby poly halogenation on carbon, nearby polar groups, and intramolecular hydrogen-bonding. These other factors also contribute in a positive manner to Eq. (3.40).

58. ARE VALUES FOR K_{ow} USEFUL TO PREDICT BREAKTHROUGH IN RP-SPE?

The capacity factor for a RP-SPE cartridge when pure water is used, k'_w , discussed earlier, has been found to be closely related to K_{ow} and is related by (72, 73):

$$\log k'_w = 0.988 \log K_{ow} + 0.02$$

Hence, merely obtaining octanol-water partition coefficient values from various tabulations in the literature can yield a predictive value for k'_w because we have shown that k'_w is directly related to the breakthrough volume, V_r .

59. WHERE CAN ONE OBTAIN K_{ow} VALUES?

The resources cited are available in most university libraries in order to find these values as well as to obtain good introductions. Leo et al. published a comprehensive listing (74) and followed this with a more systematic presentation (75). Even earlier, Hansch et al. published their findings (76). Lyman et al. have published a handbook on the broad area of physico-chemical property estimations (77). These reference sources also provide detailed procedures for calculating and for estimating K_{ow} .

60. CAN BREAKTHROUGH VOLUMES BE DETERMINED MORE PRECISELY?

A more precise way to determine the breakthrough volume for a particular analyte on a given SPE sorbent, V_r , is to use reversed-phase HPLC. The SPE sorbent is efficiently packed into a conventional HPLC column. The analyte of interest is injected into the instrument while the mobile-phase composition is varied. This is accomplished by varying the percent organic modifier such as acetonitrile or methanol and measuring the differences in analyte retention time. Extrapolation of a plot of k' versus % organic modifier to zero % modifier yields a value for the capacity factor for that analyte at 100% water. This capacity factor is represented by $k'_{w,\text{SPE,HPLC}}$, where the subscript refers to 100% water. The breakthrough volume is related to the capacity factor in 100% water according to

$$V_r = V_0(1 + k'_{w,\text{HPLC}})$$

V_0 is the void volume of the SPE column and k'_w (SPE, HPLC) is the capacity factor of the analyte eluted by water. Even though this capacity factor should theoretically be the same for any technique used, as V_0 is found by knowing the porosity of the sorbent and the geometric of the SPE column or sorbent bed in the cartridge. The capacity factor of a given analyte, $k'(\text{SPE, HPLC})$, is generally obtained from HPLC. From an HPLC chromatogram, $k'(\text{SPE, HPLC})$ is obtained by taking the ratio of the difference in retention time between an analyte and its void retention time to the void retention time. Expressed mathematically,

$$k'_{\text{HPLC}} = \frac{t'_R - t'_0}{t'_0}$$

where t'_R represents the retention time for the analyte of interest under the HPLC chromatographic conditions of a fixed mobile-phase composition. t'_0 represents the retention time for an analyte that is not retained on the stationary phase. t'_0 also relates to the void volume in the column.

$k'_{w,\text{SPE,HPLC}}$ is obtained by extrapolation of a plot of k' versus % MeOH to zero. This plot is shown in Figure 3.27. A linear relationship exists between capacity factor, $k'(\text{SPE, HPLC})$ and the % MeOH concentration for a specific organic compound in HPLC. Because $k'_{w,\text{SPE,HPLC}}$ has also been shown to be related to the octanol–water partition coefficient, K_{ow} , $k'_{w,\text{SPE,HPLC}}$ and, hence, V_r can be obtained for a given RP-SPE sorbent by this approach.

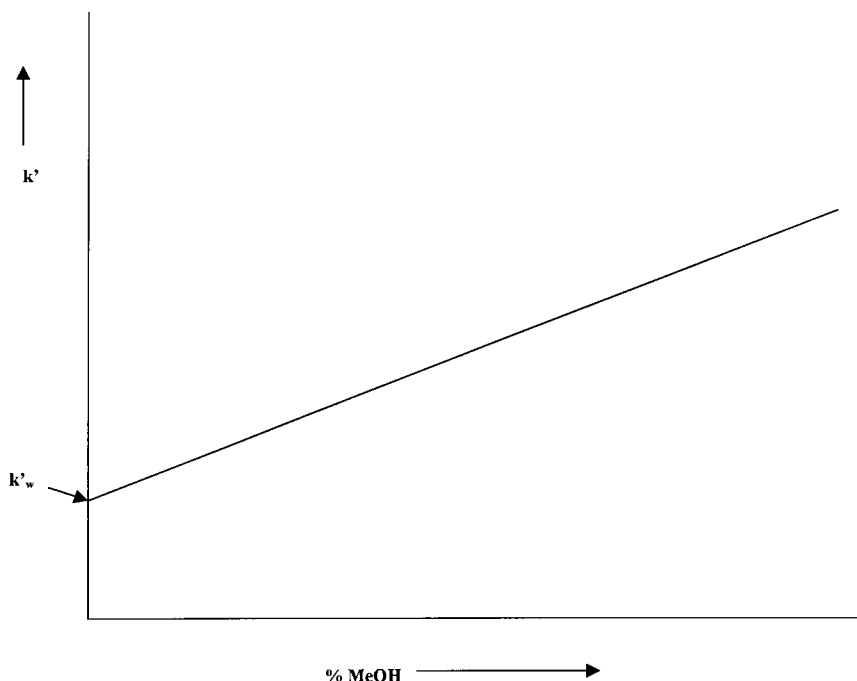


FIGURE 3.27 Plot of HPLC capacity factor versus the percent methanol in the mobile phase.

61. HOW DOES SPE RELATE TO TEQA?

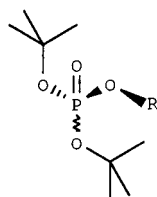
Because environmental matrices are largely air, soil, and water of some sort, the objective in TEQA is to isolate and recover a hydrophobic organic substance from a more polar sample matrix. RP-SPE can directly remove organic contaminants and represents the dominant mode of SPE most relevant to TEQA. Normal-phase SPE (NP-SPE), also has a role to play in TEQA. NP-SPE has served as an important cleanup step following LLE using a nonpolar solvent to remove polar organic interferences. This was accomplished in large-diameter glass columns instead of the familiar barrel type of SPE cartridge. Hydrophobic organic contaminants found in the environment can be easily transferred from the native matrix to the RP-SPE sorbent using procedures that will be discussed below.

Since 1985, the analytical literature has been replete with articles that purport good to excellent recoveries of various organic compounds that have been isolated and recovered by applying RP-SPE techniques. The rise in popularity of RP-SPE between 1985 and the present has served to

limit the growth of both LLE and supercritical fluid extraction (SFE). Hydrophobic organic contaminants are partitioned from the environmental matrix to the surface layer of the organo moiety. The contaminants are then eluted off of the sorbent using a solvent with the appropriate polarity. This eluent is then introduced into the appropriate determinative technique such as a gas or liquid chromatograph.

Reversed-phase SPE is applied principally in two areas of TEQA. The first application is in the determination of organochlorine pesticides (OCs) in connection with the determinative technique of GC using a chlorine-selective detector. The second application is in the determination of priority pollutant semivolatile organics that are adequately recovered from drinking water. Off-line RP-SPE coupled to a determinative technique such as GC using an element-selective detector is a very powerful combination with which to achieve the objectives of TEQA. To illustrate, this author's study of various organophosphorous pesticides (OPs) from spiked water using RP-SPE will now be discussed.

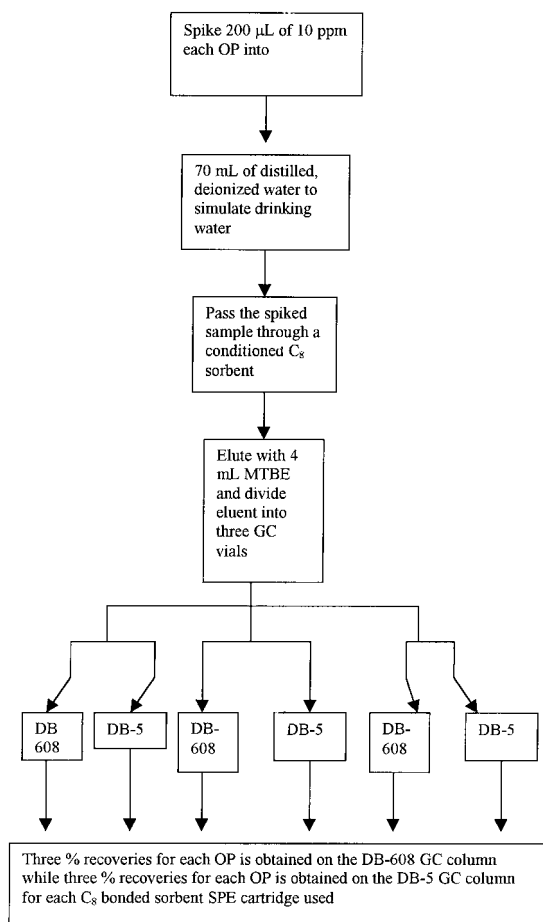
Organophosphorous pesticides are used primarily as insecticides, and in mammals, they act as cholinesterase inhibitors. A number of OPs are listed as priority pollutants. EPA Method 8141A from SW-846 Revision 1, September 1994 is an analytical method that uses LLE coupled to GC with a phosphorus selective detector and a megabore-capillary column to separate and quantitate some 49 individual OPs. The method is highly detailed and points out numerous challenges to be encountered during implementation. OPs are all structurally related in that a phosphate, thiophosphate, or dithiophosphate has been esterified. Either methyl or ethyl esters occupy two of the three functional groups around central pentavalent phosphorus, whereas a complex organo moiety is esterified to the third functional group. The generalized structure is as follows:



Generalized OP Molecular
Structure

This complexity gives rise to a wide range of compound polarity. The polarity is largely governed by the nature of this third esterified functional group.

The multisample nature of the conventional SPE vacuum manifold enabled enough replicate extractions to permit a statistical evaluation to be accomplished. A multiinjection procedure using a liquid GC autosampler enabled enough replicate injections to be made as well. This data were plentiful enough to enable equations first introduced in Chapter 2 to be used. The following flow chart describes part of a multisample/multi-injection study using two dissimilar GC megabore-capillary GC columns while spiked samples are passed through a conditioned octyl-bonded silica, C_8 :



Four other replicate SPE extractions/elutions were conducted to yield a total of five replicate SPEs. A similar study was also conducted using a octadecyl-bonded silica, C_{18} . Each RP-SPE cartridge contained 200 mg of

sorbent and each cartridge was eluted with two successive 500- μ L aliquots of methyl *tert*-butyl ether (MTBE). The eluent volume was then adjusted with MTBE to a total volume of 4 mL. This enabled triplicate injection with one injection per GC vial to be accomplished. Thus, triplicate injection for each SPE eluent combined with 5 replicate SPEs per sorbent type led to 15 replicate determinations of the percent recovery for each chromatographically resolved OP studied.

Such a plethora of data from the design used above enabled Eq. (2.33) to be used to first calculate a pooled standard deviation for triplicate injections made for each of five SPEs conducted. With mean area counts and corresponding standard deviations about the mean area account for each OP, Eq. (2.30) can be used next to calculate the mean percent recovery over spiked samples and controls. Relative standard deviations (RSDs) were obtained by dividing the standard deviation by the mean percent recovery. RSDs can be propagated between spiked samples and controls using Eq. (2.31) to yield a more realistic RSD and converted to coefficient of variations using Eq. (2.32). Table 3.11 is a summary of the mean percent recoveries and the corresponding statistical evaluation according to Eqs. (2.30)–(2.33) (78). The C₁₈ sorbent gave a larger overall percent recovery when compared to the recoveries using the C₈ sorbent. One OP, dimethoate, exhibited a relatively low percent recovery. This is not that surprising due to the presence of the polar *N*-methylamido-esterified functional group. All other OPs studied have nonpolar esterified functional groups and percent

TABLE 3.11 Comparison of the Percent Recoveries of Various OCs from Spiked Water Between a C₈ and a C₁₈ Chemically Bonded Silica Sorbent

OP	C ₈ % Recovery (coeff of var)	C ₁₈ % Recovery (coeff of var)
TEPT	77.6(3.9)	94.0(3.1)
Dichlorovos	42.0(3.0)	56.9(2.6)
Ethoprop	85.0(2.1)	92.0(1.9)
Phorate	80.1(2.5)	54.6(2.2)
Diazinon	83.5(2.3)	89.3(2.4)
Dimethoate	4.1	5.0
Methyl parathion	89.0(3.0)	96.3(2.7)
Parathion	83.6(2.5)	94.3(2.9)
Tokuthion	41.4(2.6)	70.4(3.0)
Famphur	90.0(2.3)	99.9(3.3)
EPN	61.8(2.7)	86.3(3.3)
Azinphos methyl	92.1(3.4)	97.2(3.8)

recoveries were found to be more than adequate when compared to Method 8141A. The coefficient of variation in the mean is consistently below 5% among 11 of the 12 OPs selected for study. The application of the Leo Fragment method, as discussed earlier, would make for an interesting estimate of the octanol–water partition coefficient for each of these OPs.

We now discuss this author's earlier experience with RP-SPE using selected OCs. This discussion will serve to both illustrate the technique and close out this aspect of sample preparation.

62. SHOULD I USE RP-SPE TO MEASURE OC IN SOIL AND WATER?

This author's experience in applying RP-SPE to spiked water answers the question in the affirmative. OCs that exhibit insecticidal properties and were in widespread use early in the twentieth century are easily isolated from water and recovered by solvent elution. The majority of OCs that were used as insecticides are relatively high-molecular-weight semivolatile organic molecules having a complex structure. The original approach adopted by the EPA was encompassed in Method 608 as applied to wastewater and required LLE followed by a NP-SPE cleanup using Florisil and determination using packed-column GC–ECD (electron-capture detector). This method could monitor some 17 OCs. With selective use of elution solvents off of the Florisil cleanup column, some fractionation, hence separation of OCs from multicomponent OCs such as toxaphene and Aroclors (mixtures of polychlorinated biphenyl congeners commercially manufactured), could be achieved. The replacement of conventional LLE with Florisil cleanup with a direct isolation of OCs from samples of environmental interest using RP-SPE represented a clear alternative to EPA Method 608. When RP-SPE is used in combination with high-resolution capillary GC–ECD, a much more robust and powerful analytical method emerged. Samples could be prepared and analyzed much faster and the increase in GC resolution afforded by capillary columns enabled a greater degree of delineation among all OCs of interest. This author has always been rather taken back by how efficiently these OCs can be removed from spiked water samples. Of the some dozen or so OCs that are routinely monitored in drinking water (EPA Method 508), wastewater (EPA Method 608), and solid waste [EPA Method 8081A (Revision 1, December 1996)], I chose three that are most representative of all of these OCs, namely lindane or γ -BHC, endrin, and methoxychlor. Of these three, methoxychlor is still in use.

63. CAN LINDANE, ENDRIN, AND METHOXYCHLOR BE ISOLATED AND RECOVERED USING RP-SPE?

Yes, and the data from one such study by this author is now discussed. Lindane, being a hexachlorocyclohexane, has a higher aqueous solubility than hexachlorobenzene. The gamma isomer, γ -BHC, is some 10 times more soluble than the alpha or beta isomer. Lindane (γ -BHC) represents the class of chlorinated aliphatic hydrocarbons with an S_{aq} of between 7.5 and 10 ppm. Endrin is a member of the cyclodiene insecticides, possessing the characteristic "endomethylene bridge" structure. The S_{aq} for endrin is 0.23 ppm, which is similar to that for aldrin, dieldrin, and heptachlor epoxide but greater than heptachlor and chlordane. Endrin is known to partially break down to endrin aldehyde and to endrin ketone in packed GC columns. It is not much of a problem when capillary columns are used. Methoxychlor, a substituted diphenyl trichloro ethane similar to DDT, has a S_{aq} of between 0.1 and 0.25 ppm and it is about 100 times more soluble in water than is DDT. Using ethyl acetate as an elution solvent and some of the early chemically bonded silica, of 60 Å pore size and 40 μm irregular particle size (Separalyte, Analytichem International), this author conducted a series of systematic studies of lindane, endrin, and methoxychlor (L,E,M). The work was focused on a sample matrix consisting of distilled deionized water (DDI) and the analysis was performed on a gas chromatograph that used a packed column and electron-capture detector.

One such study is reported here. Nine cartridges were packed with an octyl-bonded silica, C_8 Separalyte. The mass of the sorbent packed in each cartridge varied from a low of 20 mg to a high of 320 mg. In each cartridge, after methanol conditioning, a spiked aqueous sample was passed through the packed bed under a reduced-pressure SPE manifold. Then 8.8 μL of a methanolic reference standard containing L, E, and M each at a concentration of 100 ng/ μL (ppm) was added to approximately 60 mL DDI. This spiking gave a concentration level in the aqueous sample of 15 pg/ μL (ppb). This concentration level is considered low enough so as to approximate the realm of TEQA. After the sample was passed through the nine replicate RP-SPE cartridges, two 500- μL aliquots of ethyl acetate were used to elute the sorbed analytes into a 1-mL receiver volumetric flask. The contents of the volumetric flask were quantitatively transferred to a 2-mL GC autosampler glass vial and 2 μL of this eluent were injected into a GC that contained packed chromatographic column. Only one injection per GC vial was made in this particular study so that variation in the % recovery reflects only the random error associated with the RP-SPE process only. Table 3.12 lists the % recovery for all three OCs from each of the nine cartridges. The concen-

TABLE 3.12 Percent Recoveries of OCs Using a C₈-Bonded Silica Sorbent from Spiked Water

No. of mg sorbent	Lindane	Endrin	Methoxychlor
115	94.3	111	93.2
291	97.1	87.3	80.8
225	106	133	107
241	100	111	100
98	100	122	100
20	100	106	93.2
52	77.1	87.3	86.3
130	97.1	113	93.2
320	100	95.2	93.2

Note: 8.8 μ L of 100 ppm each, lindane, endrin and methoxychlor, dissolved in methanol was added to approximately 60 mL of distilled, deionized water. The spiked sample was passed through the cartridge, previously conditioned with methanol, and eluted with two 500- μ L aliquots of ethyl acetate. The volume of eluent was adjusted to 1.0 mL, and 2 μ L of eluent was injected into a GC.

tration of analyte that constituted a 100% recovery was calculated and not actually measured; hence, there is no contribution to the relative standard deviation from the control standard as shown in Eqs. (2.30) and (2.31). It is evident from review of the percent recovery results shown in Table 3.12 that breakthrough was not reached even in the case where only 20 mg of sorbent was taken to prepare the packed cartridge.

The mean percent recovery can be found from these nine replicate SPEs. The standard deviation in the mean percent recovery is then found using the fundamental equation for calculating standard deviations. If replicate injections per GC vial were made, a pooled standard deviation relationship as given in Eq. (2.33) would be most appropriate. From the standard deviation, a relative standard deviation or coefficient of variation is obtained and the corresponding confidence interval. For the percent recoveries shown in Table 3.12, the following statistical evaluation was obtained:

OC	Mean % recovery	RSD (%)	Conf. Interval (95%)
Lindane	96.8	8.3	6.2
Endrin	107.3	14.3	11.8
Methoxychlor	94.1	8.2	5.9

The mean percent recoveries in this replicate series of RP-SPEs are very high and represent a statement of accuracy in the measurement. A relative standard deviation of 8% or 14 % among replicate SPEs represents the precision of the method. The confidence interval states that of the next 100 SPEs, 95 of these should fall to within the interval specified. For example, if 100 additional percent recoveries using the SPE method could be performed for lindane, one could expect that 95 would fall to within $96.8 \pm 6.2\%$.

This high and reproducible percent recovery for the isolation and recovery of lindane from water strongly suggests that RP-SPE is very appropriate as a sample preparation method for this analyte. Lindane is of continued interest to environmental and toxicological scientists. One such study, discussed next, taken from the author's work, involves the isolation and recovery of lindane from homogenized myometrial tissue suspended in an aqueous matrix.

64. WAS LINDANE ISOLATED AND RECOVERED FROM A BIOLOGICAL MATRIX USING RP-SPE? IF SO, HOW WAS THIS DONE?

One hundred microliters of a methanolic solution containing 5 ng/ μ L (ppm) lindane was placed in a 1-mL volumetric flask half-filled with iso-octane while 100 μ L of the same lindane reference standard was added to approximately 70 mL of distilled, deionized water (DDI). One microliter of the former solution containing 500 ng of lindane was injected into a GC and the resulting peak area served to define a control that represents a 100% recovery of lindane. One microliter of the 1 mL eluent from performing the RP-SPE of the spiked DDI was also injected into the same instrument and the resulting peak area served to define the spiked recovery sample. In this study, quadruplicate injections of the control yielded a mean concentration of 400 ppb lindane from interpolation of the least squares calibration curve. Seven replicates of the eluent from the spiked DDI sample were injected and a mean concentration of 406 ppb lindane was obtained from interpolation of the same calibration curve. Equation (2.30) was used to find a 106% recovery of lindane. Equation (2.31) was used to find the relative standard deviation for the % recovery by propagating error between spiked sample and control. A complete result can be given by stating both the accuracy, 106% recovery, and the relative standard deviation of 15.6%. Myometrium samples were then prepared and lindane appeared as expected. This work showed that lindane could easily be isolated and recovered from an aqueous matrix and confirmed the earlier work on lindane isolation and recovery discussed previously. One would be led to believe that this is a robust sample preparation method for

lindane because it can be reproduced with confidence. Thus, a subsequent request for additional sample analyses does not require the extensive QC and should merely report the analytical results.

65. WHAT DOES A SAMPLE ANALYSIS REPORT LOOK LIKE?

In addition to a tabular format for the determination of lindane in each of the myometrial tissue samples submitted, a method summary should be included. A one paragraph method summary serves to inform the reader as to how the samples were handled once they arrive in the analyst's laboratory. The summary should also provide a brief overview of the sample preparation and a brief description of the determinative technique used. The following report illustrates these concepts.

REPORT ON THE QUANTITATIVE DETERMINATION OF LINDANE IN MYOMETRIAL TISSUE SUSPENDED CELLS IN SALINE

Summary of Method

The entire contents (1 mL) of sample that was received by the client was refrigerated upon receipt until the sample was prepared for analysis. Upon thawing, the entire contents of each sample was added to a reservoir that contained approximately 70 mL of distilled deionized water. This aqueous solution was passed across a previously conditioned octadecyl-bonded silica sorbent (C_{18} RPSiO₂). The retained analyte was eluted off of the sorbent with two 500- μ L aliquots of pesticide-residue-grade iso-octane. The iso-octane eluent was then passed through a second SPE cartridge. This second cartridge was packed with approximately 0.5 g anhydrous sodium sulfate. The volume of eluent which now contained the recovered analyte was adjusted to a final volume of 1.0 mL using a volumetric flask. Three microliters of this eluent was injected via autosampler into a Autosystem Gas Chromatograph (Perkin-Elmer) incorporating an electron-capture detector. A 30-m $\times$ 0.32-mm capillary GC column containing DB-5 (J & W Scientific) was used to separate the organics in the eluent. This instrument is abbreviated C-GC-ECD to distinguish it from other gas chromatographs in our laboratory. The column was temperature programmed following injection from 200°C to 270°C at a rate of 10°C/min. The C-GC-ECD is connected to a 600 Link (PE-Nelson) Interface Module. This interface, in turn, is connected to a 386 personal computer. This PC uses Turbochrom (PE-Nelson) software for data acquisition, processing, and control. A method specific for lindane was written and one peak was identified within a 3%

relative time interval (retention time window). A retention time, $t[R]$, of 1.8 min was consistently reproduced using autosampler injection.

Calibration

A series of calibration or working standards were prepared from the methanolic stock solution containing lindane. This stock solution was prepared by carefully weighing out pure solid lindane on an analytical balance. These standards were injected into the C-GC-ECD from lowest to highest lindane concentration. The peak at 1.8 min was identified as a reference peak in the Turbochrom software and a narrow $t[R]$ window was defined around the $t[R]$ of the apex of the peak. A calibration curve was constructed using a least squares regression algorithm in the software. A correlation coefficient of 0.9990 was obtained.

Sample Analysis

The sequence within which the samples were run after calibration was established was created by using the Sequence File Editor within Turbochrom. Samples are injected via autosampler according to the instructions in the Sequence File. After the sequence is completed, a Summary File was created and the analytical results are reported within this summary format. In the Summary File, for each sample analyzed, the following is given:

- (a) The sample number
- (b) The concentration of lindane in ppb for 1.0 mL eluent, interpolated from the external standard mode of instrument calibration
- (c) The retention time for lindane, $t[R]$, in minutes
- (d) The integrated peak area, in μ volts-seconds

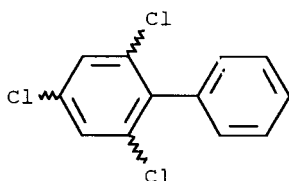
The reported concentration is that for each eluent and should be multiplied by the eluent volume (in this case, 1.0 mL) to obtain the number of nanograms of lindane found. The number of nanograms found divided by the volume of the aqueous sample used in RP-SPE gives the reported concentration in ppb for lindane. The reported concentration should be divided by the volume of the sample to obtain the reported concentration in ppb for lindane. The reported concentration should also be divided by the percent recovery expressed as a decimal in order to find the true and final concentration of lindane in the original sample.

Method Evaluation

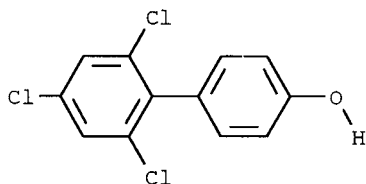
Lindane is efficiently recovered from aqueous matrices with percent recoveries between 75% and 100% using RP-SPE. The biological sample closely approximates an aqueous matrix.

66. DOES IT MATTER WHICH ELUTION SOLVENT IS USED IN RP-SPE?

Answers to this can be given with a yes and no and a qualifying statement that it might depend on the polarity of the retained analyte. With a series of analytes that have similar octanol–water partition coefficients, differences in eluting solvent polarity may not significantly influence analyte recovery. If breakthrough studies are not performed as discussed earlier, one never knows whether low percent recoveries are due to poor sorption or to inefficient elution or solvent removal using the RP-SPE technique. This author recently had the opportunity to quantitatively determine trace concentrations of 2,4,6-trichloro-biphenyl (246TCBP) and 4-hydroxy-2,4,6-trichloro-biphenyl (4HO246TCBP) in biological matrices. Molecules from these two organic compounds of environmental interest differ in that a hydroxyl group is substituted for a hydrogen on the phenyl ring para to the second phenyl. These structures are as follows:



2,4,6-trichloro-biphenyl



4-hydroxy-2,4,6-trichloro-biphenyl

We were quite surprised to measure a zero percent recovery when we attempted to apply our method for OCs to isolate and recover 4HO246TCBP from spiked water. Our recoveries of 246TCBP using our OC method were very high. Replacing iso-octane with methanol as the eluting solvent resulted in a significant increase in percent recoveries for 4HO246TCBP. This surprising finding led us to conduct a series of systematic RP-SPE recovery studies. An aqueous spiked sample was prepared that contained both PCB congeners and two elution schemes were used. In

Scheme A, the retained analytes were eluted with methanol followed by a separate second elution with iso-octane. Two receivers were used and their final volumes were both adjusted to a 1.0-mL total eluent volume. In Scheme B, the retained analytes were eluted with iso-octane followed by methanol. The integrated peak areas (in units of $\mu\text{V}\cdot\text{s}$) for the 1 mL eluents are presented in Table 3.13. It is a good assumption that the response factors for both congeners are near one another because the substitution of a hydroxyl for hydrogen in the phenyl ring contributes little or nothing to the ECD response. Eluting first with two 500- μL aliquots of methanol recovers more 4HO246TCBP when compared to 246TCBP, whereas the second elution with iso-octane recovers nearly equal amounts of both congeners. Thus, Scheme A reflects incomplete recovery of the congeners with two 500- μL aliquots of methanol. Eluting first with two 500- μL aliquots of iso-octane recovered even more 246TCBP when compared to MeOH yet recovered no 4HO246TCBP! This observation was consistent with our preliminary finding discussed earlier. Upon eluting with MeOH via Scheme B, a relatively small amount of 246TCBP was recovered whereas a relatively large amount of 4HO246TCBP was recovered. The percent recoveries for 4HO246TCBP were similar between Schemes A and B when MeOH was used to elute off the retained analyte.

Another illustration from the author's experience is the differences in percent recovery when hexane is used versus methyl *tert*-butyl ether (MTBE), to elute off of a C18 sorbent. Two different aqueous matrices were spiked with the OPs diazinon and malathion. In one series of experiments, two 500- μL aliquots of hexane were used to elute off of the cartridge followed by two 500- μL aliquots of MTBE eluted off the same cartridge. The same experiment was performed on a second aqueous matrix that was more acidic by virtue of adding potassium dihydrogen phosphate (KH_2PO_4). The results are shown in Table 3.14. It is clearly evident that

TABLE 3.13 Comparison of Two Sample Preparation Schemes to Isolate and Recover 2,4,6-Trichlorobiphenyl and 4-hydroxy-2,4,6-trichlorobiphenyl Using RP-SPE

	246TCBP	4HO246TCBP
Scheme A		
Elute with MeOH, mean of four SPEs	137,026	218,526
Elute with iso-octane, mean of four SPEs	40,413	58,259
Scheme B		
Elute with iso-octane, mean of four SPEs	210,234	0
Elute with MeOH, mean of four SPEs	24,825	194,002

TABLE 3.14 Percent Recoveries for Diazinon and Malathion Using Hexane and MTBE as Elution Solvents in RP-SPE

	Diazinon	Malathion
Matrix: 10% NaCl (DDI)		
1. Elute with hexane, mean of 3 SPEs	20	0
%RSD	18.3	—
2. Elute with MTBE, one SPE	118	112
Matrix: 10%NaCl, 0.2M phosphate buffer		
1. Elute with hexane, mean of 3 SPEs	20	0
%RSD	14	—
2. Elute with MTBE, one SPE	112	98

the more polar MTBE more effectively removes both OPs from the hydrophobic sorbent than the less polar hexane. This fact has perhaps more to do with the polarity of OPs and the nature of the sorbent-analyte-solvent “triangle” as discussed earlier in this chapter.

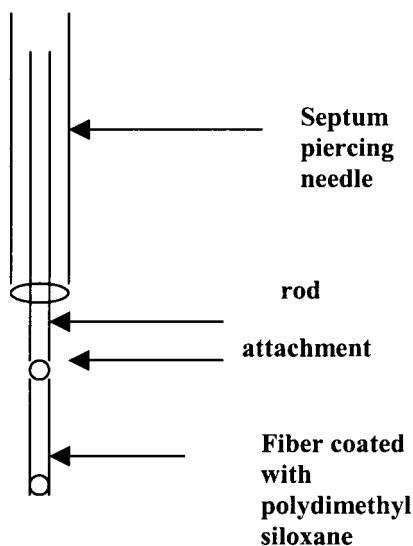
67. WHAT CAN WE CONCLUDE ABOUT RP-SPE ?

Reverse-phase SPE as a sample preparation technique is low cost, effective, and continues to gain popularity as regulatory restrictions lift over time with respect to TEQA. Several reviews published earlier provide a good summary of SPE-related research as applicable to TEQA. Font et al. has reviewed developments in water pollution analysis for PCBs (79) and earlier published a review applicable to multiresidue pesticide analysis of water (80). The use of matrix solid-phase dispersion as applied to determining toxic wastes in biological tissue from aquatic species provides a good review of this extension of RP-SPE (81). A comprehensive study that convincingly demonstrates that RP-SPE has an important role to play in the analysis of human and bovine sera was published earlier (82). We now consider a sample prep technique developed during the early 1990s that relates to SPE and some still call it solventless SPE, namely solid-phase microextraction.

68. WHAT IS SOLID-PHASE MICROEXTRACTION?

One way to answer this question is to state that solid-phase microextraction (SPME) is to the capillary GC column what SPE is to the HPLC column. For SPE, consider taking a portion of the HPLC column packing, increas-

ing the particle size, and allowing for a wider distribution of the particle size; then pack this material into a cartridge and use this for sample prep. For SPME, on the other hand, coat a fused-silica capillary column on the outside instead of the inside and use this for sample prep. It remained for Janusz Pawliszyn at the University of Waterloo who understood the limitations of SFE and SPE and was first to modify a 7000 Series (Hamilton) liquid handling microsyringe by coating polydimethylsiloxane on a fine rod such as fused-silica fiber. (See Ref. 83 for two of the pertinent articles published by Pawliszyn and co-workers.) SPME is also *solventless* in that a sorbed analyte is easily thermally desorbed off of the coated fiber in the injection port of a gas chromatograph. The modified microsyringe of Pawliszyn has given way to the commercial SPME device that incorporates a retractable fiber and is manufactured and marketed by Supelco. The essential design of a SPME sampling device is shown in the following schematic:



The SPME sampling device can be immersed directly into an aqueous sample such as groundwater for a finite period of time, then withdrawn, the fiber and rod retracted back into the needle, brought to the hot-injection port of a gas chromatograph, and then inserted into the septum, the fiber and rod extended into the injection port for a finite period of time in which thermal desorption is accomplished. For analysis of aqueous samples for VOCs, the fiber is inserted into the headspace, sampled, retracted, and then injected directly into the injection port. For solids, wastewater, sludge, etc. this headspace technique is appropriate provided that analytes can partition

into the headspace from these “dirty” sample matrices. The rate of SPME depends on the mass transport from a matrix to the coating. The effectiveness of mass transport depends on the following:

1. Convective transport in air or liquid
2. Desorption rate from particulates that might be present in the sample
3. Diffusion of analytes in the coating itself

For direct SPME sampling with agitation, convective effects can be minimized. In the absence of particulates, the mass transport rate is determined by diffusion of analytes into the coating. For gaseous samples, the rate of mass transport is determined by diffusion of the analyte into the coating and equilibrium can be achieved in less than 1 min. For aqueous samples, vigorous agitation of the sample is necessary. A common technique is to simply stir the sample with a magnetic stirrer. In this case, it takes much longer for the analyte to diffuse through the static layer of water that surrounds the fiber. Pawliszyn has articulated the challenge for SPME as follows (83):

For volatile compounds, the release of analytes into the headspace is relatively easy because analytes tend to vaporize once they are dissociated from their matrix. For semivolatile compounds, the low volatility and relatively large molecular size may slow the mass transfer from the matrix to the headspace and, in some cases, the kinetically controlled desorption or swelling process can also limit the speed of extraction, resulting in a long extraction time. When the matrix adsorbs analytes more strongly than the extracting medium does, the analytes partition poorly into the extraction phase. Because of the limited amount of the extraction phase in SPME (as in SPE), the extraction will have a thermodynamic limitation. In other words, the partition coefficient, K , is too small, resulting in poor sensitivity. If the coating has a stronger ability to adsorb analytes than the matrix does, it is only a matter of time for a substantial amount of analytes to be extracted by the fiber coating and only kinetics plays an important role during extraction. One of the most efficient ways to overcome the kinetic limitation is to heat the sample to higher temperatures, which increases the vapor pressure of analytes, provides the energy necessary for analytes to be dissociated from the matrix, and at the same time speed up the mass transport of analytes.

It has been demonstrated mathematically that it is not necessary for the analyte to reach thermodynamic chemical equilibrium to perform TEQA

using the SPME device. Let us consider this in more detail. We start by defining the partition coefficient $K_{fs(\text{SPME})}^i$ for analyte i between the fiber, denoted by f , and a sample, denoted by s , as follows:

$$K_{fs(\text{SPME})}^i = \frac{C_f}{C_s} = \frac{n/V_f}{C_0 - (n/V_s)} \quad (3.44)$$

where

n : amount of analyte i adsorbed on the SPME polymer film (in moles)

V_f : volume of coating on SPME fiber

V_s : volume of sample

C_0 : initial concentration of the i th analyte in the sample

C_f, C_s : concentration of the i th analyte in the fiber and sample, respectively, once equilibrium is reached

Solving Eq. (3.44) for n yields

$$n = \left[\frac{K_{fs(\text{SPME})}^i V_f V_s}{V_s + K_{fs(\text{SPME})}^i V_f} \right] C_0 \quad (3.45)$$

If the partition coefficient is quite large, then

$$K_{fs(\text{SPME})}^i V_f \gg V_s$$

and

$$n \cong V_s C_0 \quad (3.46)$$

Equation (3.46) states that the amount of analyte adsorbed into the fiber coating in SPME is directly proportional to the original concentration of analyte in the sample. This amount is reached after equilibrium has been attained. The term used is exhaustive SPME.

69. CAN WE QUANTITATE BEFORE WE REACH EQUILIBRIUM IN SPME?

We answer this question by considering the derivation first proposed by Ai (84). The assumption is that analyte molecules diffuse from (a) the sample

matrix to the surface of the polymer and (b) from the polymer surface to the inner layers. For a steady state, with diffusion as the rate-controlling factor, the mass flow rate of analyte molecules from the sample matrix to the SPME polymer surface would equal the flow rate from the polymer surface to its inner layers. With D_1 as the diffusion coefficient of analyte molecules in the sample matrix and D_2 as the diffusion coefficient for molecules in the polymer phase whose surface area is denoted by A and C_s and C_f are concentrations of analyte in the sample matrix and polymer film respectively, Fick's First Law can be used to give the rate-determining steps governed by

$$\frac{1}{A} \frac{\partial n}{\partial t} = -D_1 \frac{\partial C_s}{\partial x} = -D_2 \frac{\partial C_f}{\partial x}$$

By assuming that a steady-state mass transfer occurs when agitation is effectively applied, assuming that the diffusion layer is a thin film, and steady-state diffusion in this thin film is in effect, a simplified normal differential equation can be considered:

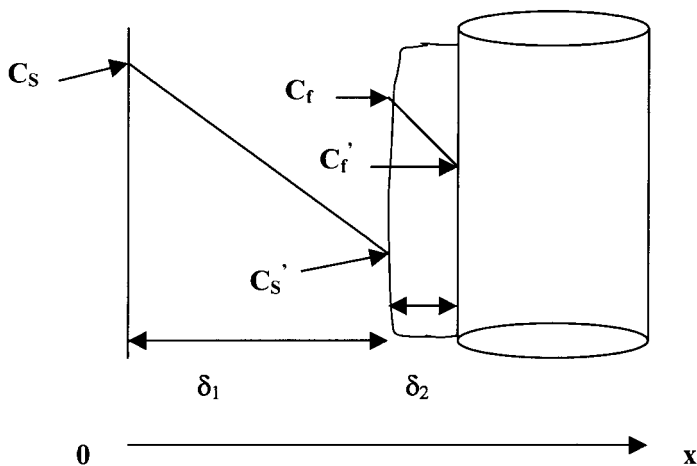
$$\begin{aligned} \frac{1}{A} \frac{dn}{dt} &= \frac{D_1}{\delta_1} (C_s - C'_s) \\ &= \frac{D_2}{\delta_2} (C_f - C'_f) \end{aligned} \quad (3.47)$$

The terms used in Eqs. (3.47) are defined as follows:

- C_s : concentration of the analyte in the bulk sample matrix
- C'_s : Surface concentration of the analyte in bulk sample matrix
- δ_1 : Diffusion layer thickness in the sample matrix
- δ_2 : Thickness of polymer film
- C_f : Analyte concentration in the polymer film at the surface
- C'_f : Analyte concentration in the polymer film in contact with the silica fiber

Shown below is a schematic of the interface of the polymer-coated silica fiber in contact with an aqueous solution. The illustration shows the diffusion layer thickness in the sample matrix and the thickness of the polymer film. The slope of the concentration gradients are shown at the interface between the bulk sample and polymer film surface. A steady-state diffusion

is assumed when the aqueous solution is effectively agitated. The concentration gradient in the SPME film is assumed to be linear:



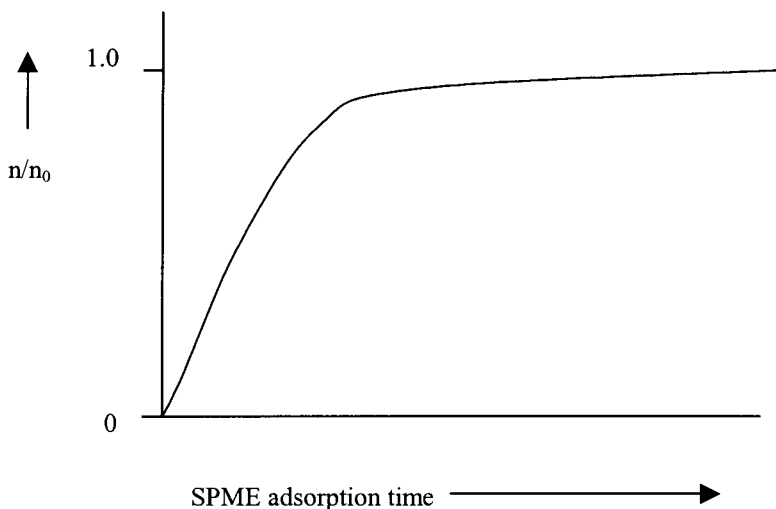
Equation (3.47) can be rearranged and integrated and within a set of boundary conditions; this ordinary differential equation can be solved for n to yield (84)

$$n = \left[1 - e^{-A(B)t} \right] \left[\frac{KV_f V_s}{KV_f + V_s} \right] C_0 \quad (3.48)$$

Equation (3.48) suggests that the number of moles of analyte absorbed depends only on the original concentration of analyte in the sample, C_0 , provided that the sampling time t remains constant between samples and the rates of diffusion remains constant between samples. This diffusion rate is reflected in the slope of the lines between C_s and C_s' and C_f and C_f' as shown above. As the sampling time gets very large (i.e., n approaches infinity), Eq. (3.48) suggests that n approaches n_0 , where n_0 is the number of moles adsorbed by the coating at equilibrium so that Eq. (3.48) reduces to Eq. (3.45)! Hence, Eq. (3.48) can be rewritten in terms of n as a function of sampling time according to

$$\frac{n}{n_0} = 1 - e^{-at} \quad (3.49)$$

where a is a constant that is composed of various mass transfer coefficients, $K_{fs(SPME)}$, V_f , and V_s . If plots are made of n/n_0 versus SPME adsorption or sampling time, curves such as the following one for a given analyte are expected:



In a second paper, Ai developed a theoretical treatment for HS-SPME (85). Lakso and Ng used SPME to isolate nerve gas agents such as Sarin from drinking water and observed similar uptake versus adsorption time for direct aqueous sampling (86). A plethora of SPME applications continue to appear in the literature. This author began to use SPME in pursuit of alternative sample prep methods designed to isolate and recover trace concentration levels of specific polychlorinated biphenyl (PCB) congeners, various Aroclors along with the necessary surrogates as is utilized in EPA Method 8081A.

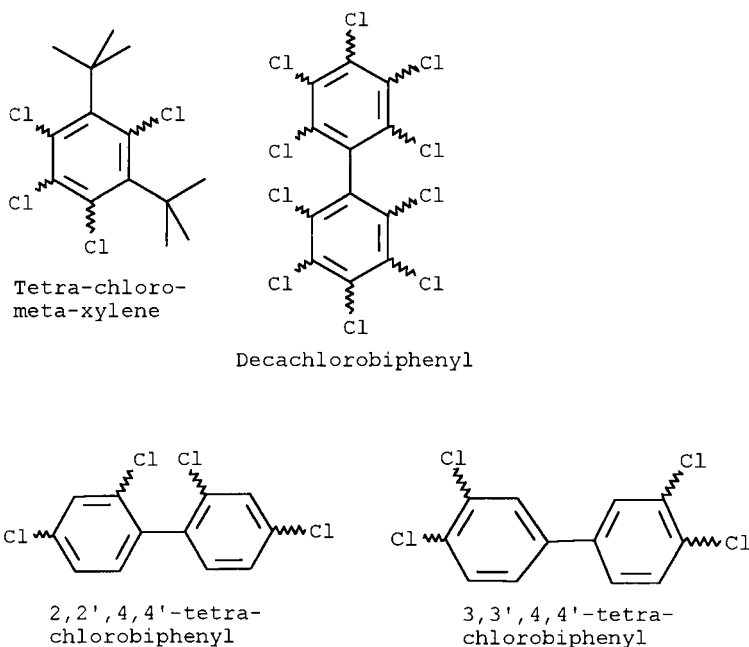
70. WHAT FACTORS NEED TO BE CONSIDERED WHEN USING SPME TO ISOLATE PCB?

The sample prep considerations are different for SPME from anything we have encountered because this is a solventless technique and requires that we consider the kinetics of sampling. Some factors are as follows:

- Sorption time for the fiber as discussed earlier
- Thermal desorption temperature of the GC injector port

- Iso-thermal column temperature and time during thermal desorption
- Chromatographic temperature program to adequately resolve peaks in minimum run time

The molecular structures of the analytes of interest are as follows:



Three chromatograms that were obtained in the author's laboratory and are overlayed for comparative purposes are shown in Figure 3.28A (87). The SPME device was used to extract the four polychlorinated organics shown earlier and thermally desorbed into the injection port of an Autosystem[®] (Perkin-Elmer) gas chromatograph that incorporated a chlorine-selective electron-capture detector (C-GC-ECD). The top chromatogram was obtained by exposing the fiber into distilled deionized water and constitutes a method blank. The method blank is a useful quality control practice in that a clean blank, as this one shows, demonstrates that the lab and analyst are free of contamination. The absence of any lab contamination suggests that the method used is free of interferences. The center chromatogram was obtained by exposing the fiber to water that had been spiked with the four polychlorinated organics. All four analytes have been extracted out of the sample

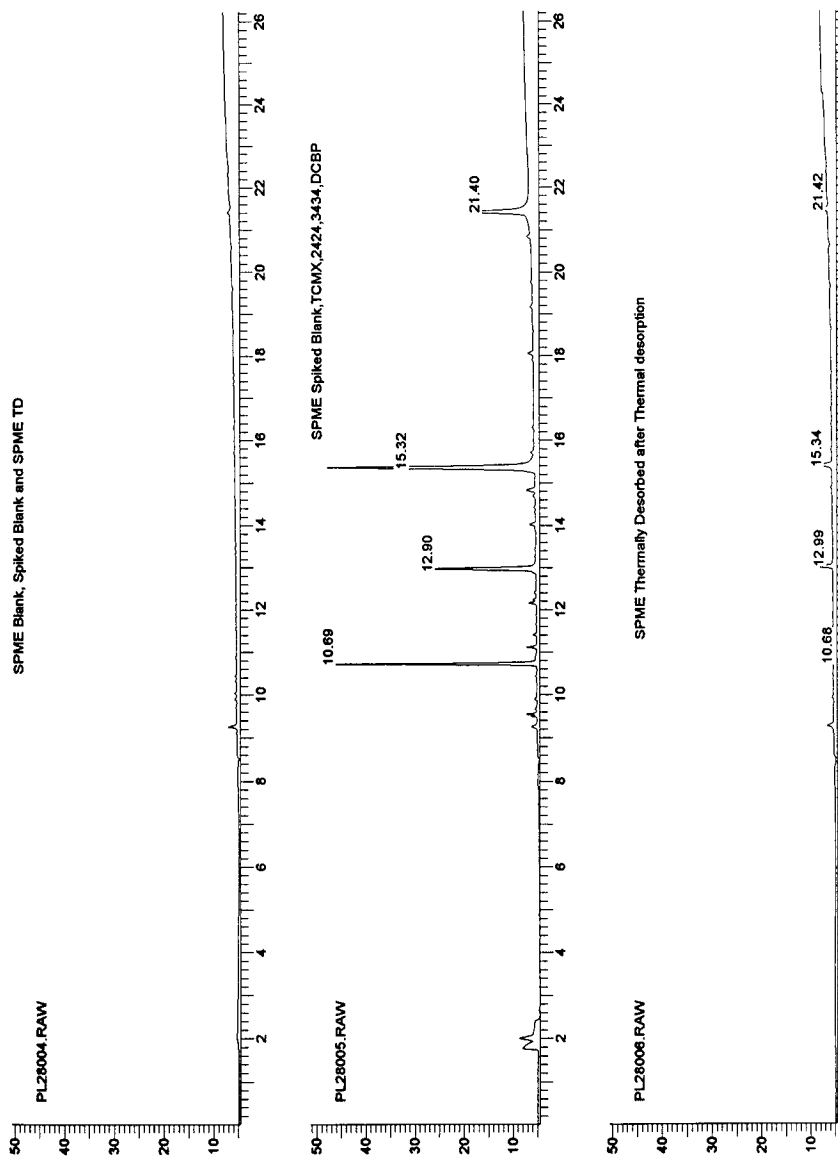


FIGURE 3.28A Comparison of three SPME/C-GC-ECD chromatograms from a blank, spiked blank and thermal desorbed blank (this and the following six figures were taken in the author's laboratory).

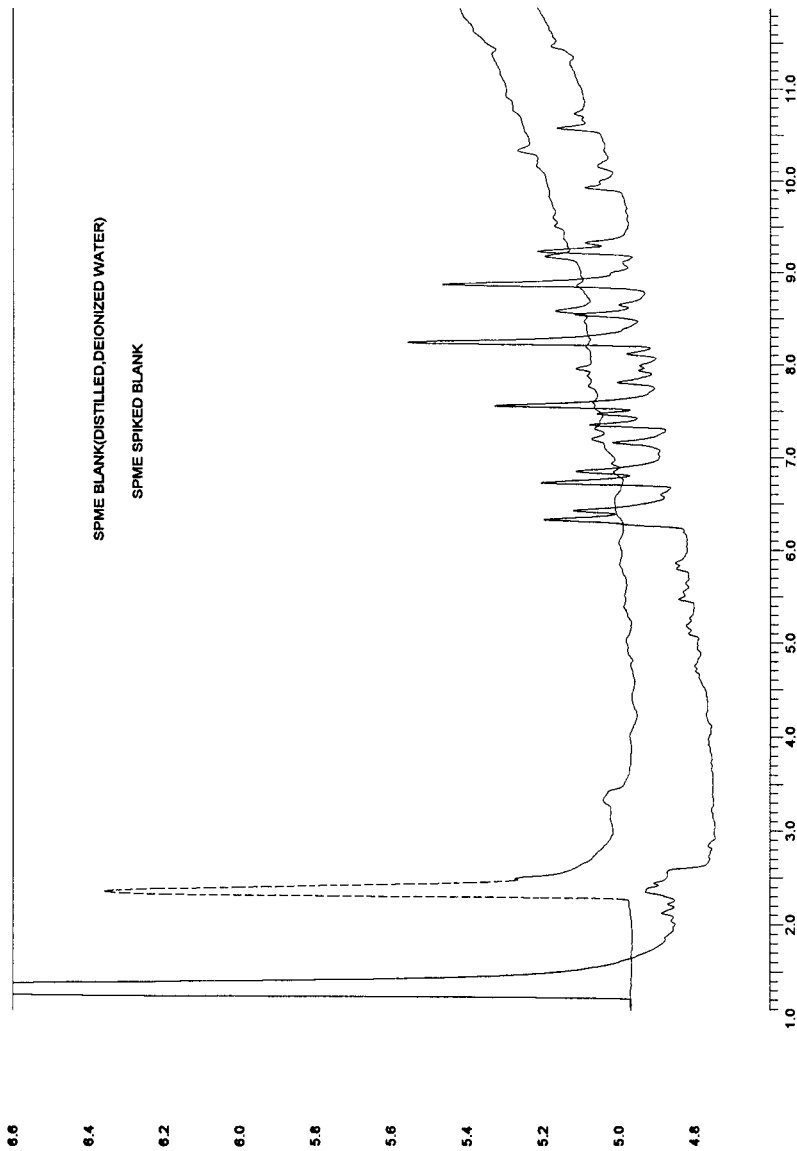


FIGURE 3.28B Overlay of SPME/C-GC-ECD chromatograms for isolating AR 1242 from a blank and a spiked blank.

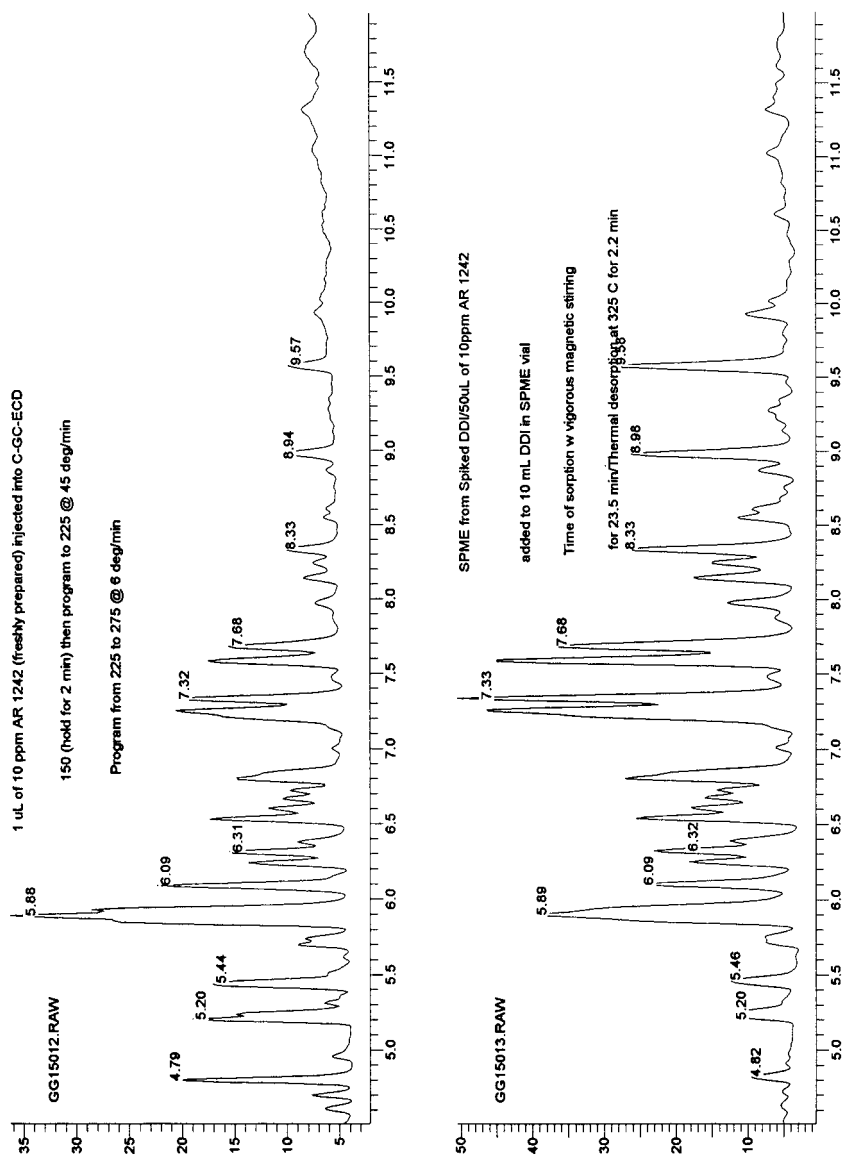


FIGURE 3.28C Comparison of a direct injection of AR 1242 and AR 1242 isolated and recovered using SPME.

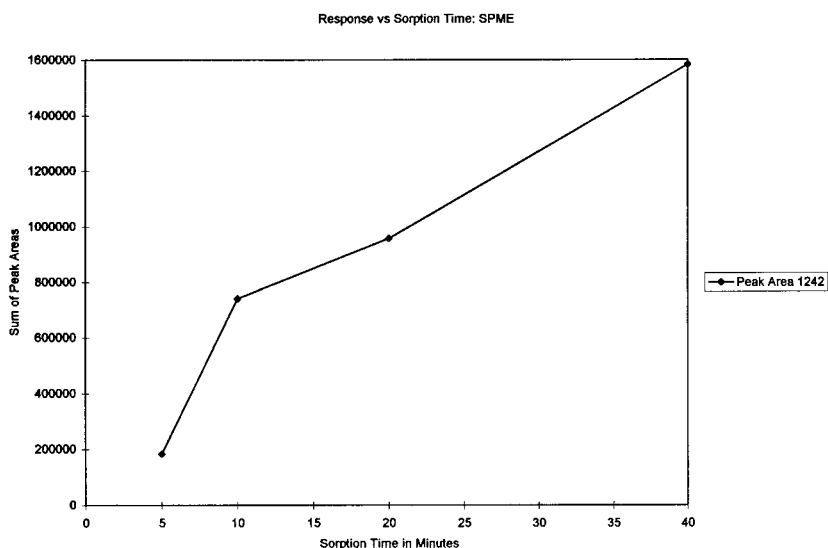


FIGURE 3.28D Plot of the sum of peak areas for AR 1242 against the sorption time for SPME; a 100 μm thick nonbonded polydimethylsiloxane fused silica fiber was used.

Component Name: "AR1242"

Date: 2/23/98 Time: 02:22 PM

Curve Parameters:

Curve #1 : 3rd Order - Incl Origin
 Weighting Factor = 1.0 (No Weighting)
 $r^2 = 0.995045$
 Calibration Curve = $(154925.175362) + (8674.964589)X + (-14.045882)X^2 + (0.010659)X^3$

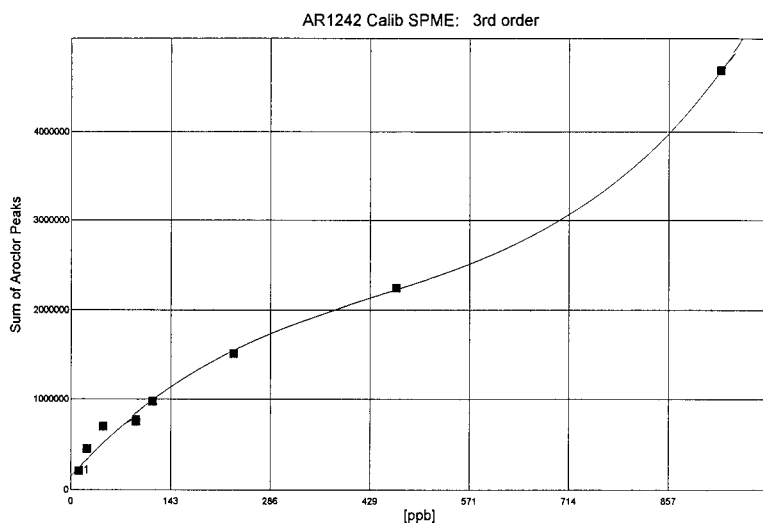
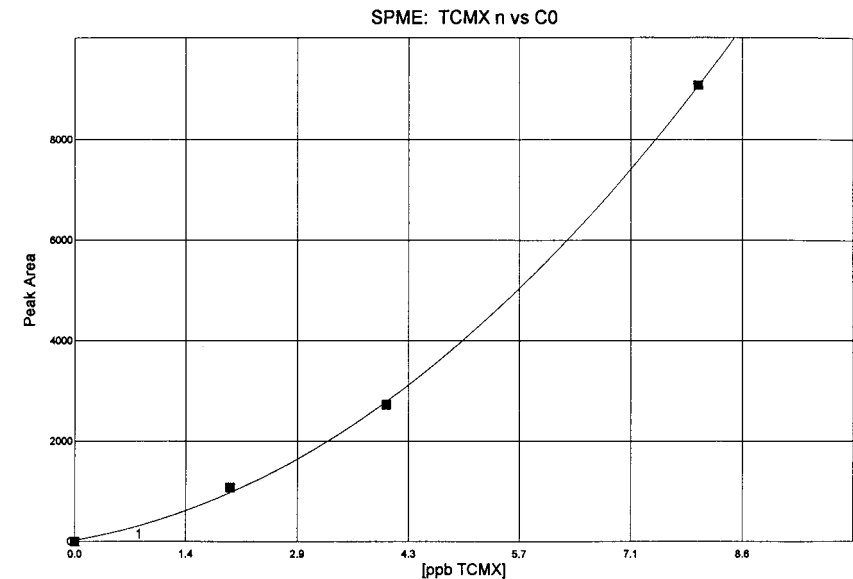


FIGURE 3.28E Plot of the sum of peak areas for AR 1242 against the concentration of AR 1242 in ppb using SPME techniques; superimposed over these points is a third order least squares regression fit. The same fiber was used here as that described for Figure 3.28D.

Curve Parameters:

Curve #1 : 2nd Order
Weighting Factor = 1.0 (No Weighting) $r^2 = 0.999689$
Calibration Curve = $(35.400000) + (254.600000)X + (109.000000)X^2$



Level Name	Observed X-Value	Calculated X-Value	Delta	%Diff.	Observed Y-Value	Calculated Y-Value	Delta	%Diff.
5	0.000000	-0.148480	0.148480	-100.00	0.000	35.400	-35.400	-100.00
5	2.000000	2.133864	-0.133864	-6.273	1075.000	980.600	94.400	9.627
6	4.000000	3.936769	0.063231	1.606	2727.000	2797.800	-70.800	-2.531
1	8.000000	8.005902	-0.005902	-0.074	9060.000	9048.200	11.800	0.130

FIGURE 3.28F Plot of the peak area for TCMX against the concentration of TCMX in ppb using SPME techniques; superimposed over these points is a second order least squares regression fit. The same fiber was used here as that described for Figure 3.28D. Calibration data is also included beneath the plot.

and were thermally desorbed into the C–GC–ECD. The separated analytes are given with their abbreviated names and GC retention times (t[R]) in the following:

Polychloro-organic	Abbreviation	t[R] Minutes after injection
Tetrachloro-m-xylene	TCMX	10.29
2,2',4,4'-tetrachlorobiphenyl	2244-TCBP	12.90
3,3',4,4'-Tetrachlorobiphenyl	3344-TCBP	15.32
Decachlorobiphenyl	DCBP	21.40

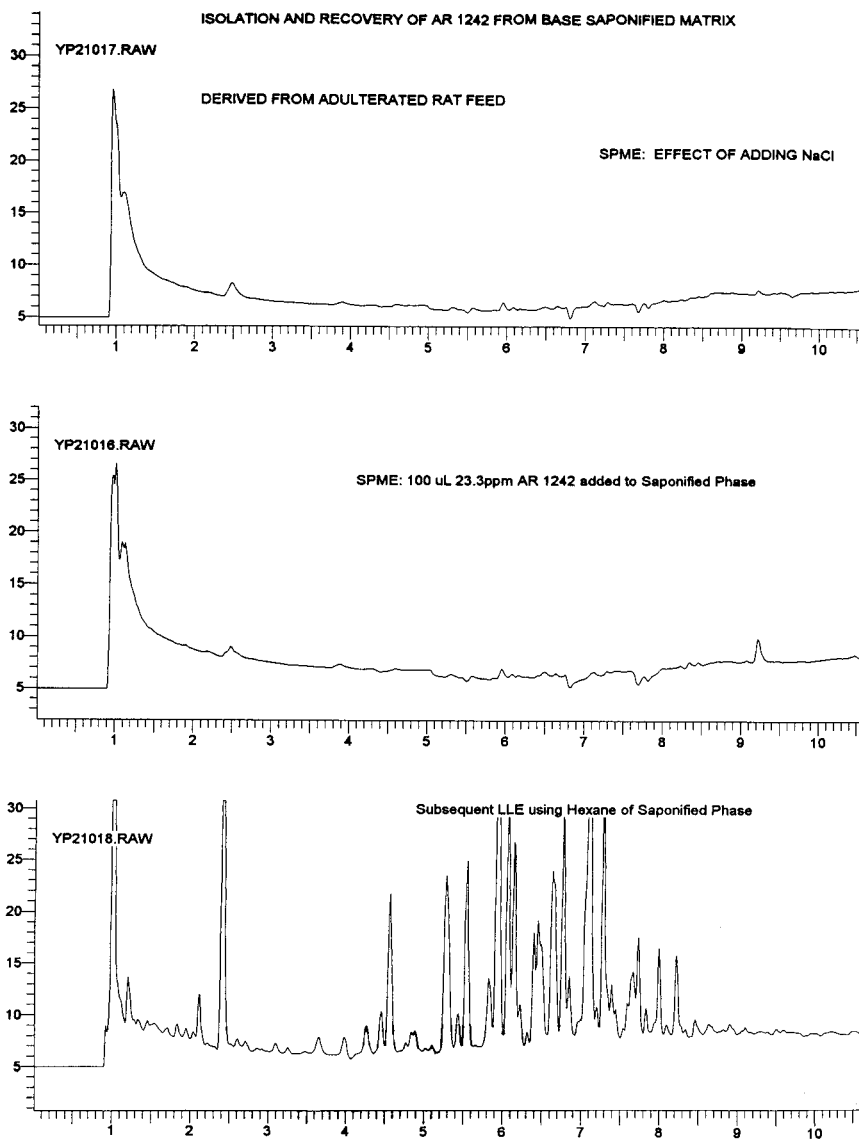


FIGURE 3.28G Comparison of SPME vs. LLE C-GC-ECD chromatograms for the isolation and recovery of AR 1242 from a base saponified aqueous sample matrix originally derived from rat feed.

The bottom chromatogram was obtained from merely injecting the already spent fiber into the hot-injection port of the C–GC–ECD a second time. Evidence of a tiny trace of each analyte is observed.

Two chromatograms are overlaid in Figure 3.28B. A comparison is shown between a spiked and unspiked aqueous sample using AR 1242. The power of SPME to preconcentrate analytes is shown in Figure 3.28C. The top chromatogram in Figure 3.28C was obtained by injection of 1 μL of a methanolic 10 ppm AR 1242 reference standard and the bottom chromatogram was obtained by applying the SPME technique to a sample that consisted of 50 μL of methanolic 10-ppm AR 1242 added to 10 mL of distilled deionized water. The sum of the numerous peaks in the chromatogram for AR 1242 provides a quantitative analysis for total Aroclor. A plot of this sum versus the SPME sorption time is shown in Figure 3.28D for four sampling times. This plot roughly follows the theoretical profile shown earlier for SPME. For a fixed sorption time and magnetic stir rate, a series of calibration standards were prepared and “SPME injected” into our C–GC–ECD. The calibration curve is shown in Figure 3.28E for some eight standards. The raw data points are fitted to a third-order polynomial as shown using Turbochrom[®] (PE-Nelson) software. This author examined the calibration and utilized the program LSQUARES (refer to the program listing in Appendix C). The results of using LSQUARES are as follows:

Concentration (ppb)	Sum of peaks from AR 1242
11.7	217,483
23.3	464,987
46.6	718,531
93.2	777,197
117	976,544
233	1,510,288
466	2,243,387
932	4,666,817

Applying LSQUARES to this calibration data yields the following:

Parameter	Value
Slope of least squares regression line (linear fit)	4548 $\mu\text{V-s/ppb}$ AR 1242
Y Intercept of least squares line	353,887
Correlation coefficient	0.9954
Y_C	674,348 $\mu\text{V-s}$
X_C	70.4 ppb
X_D	139.7 ppb
Y_D	985,686
X_{DD}	138.9ppb
ICV (low) $\pm$ confidence interval at 95%	80.2 $\pm$ 87 ppb
ICV (high) $\pm$ confidence interval at 95%	415.4 $\pm$ 87 ppb

It is clearly evident that, at least for this first attempt to simultaneously conduct sample prep and calibrate the instrument, good linearity was not achieved. Note that the initial calibration verification (ICV) as represented by as x_0 much more accurate at the higher concentration level than at the lower level. In addition, the precision as determined by the confidence interval at the 95% significance level at the lower level for the ICV is much larger than is the precision for the ICV at the higher concentration level.

A calibration plot for combined SPME/calibration for the surrogate TCMX is shown in Figure 3.28F. Again, there is a tendency for a nonlinear relationship as the concentration of TCMX increases as shown. A second-order least squares fit gave a correlation coefficient of 0.9999!

71. MIGHT THE SAMPLE MATRIX INFLUENCE SPME EFFICIENCY?

Yes, the sample matrix may significantly influence the analyte percent recovery! This became apparent to the author when a request to isolate and recover AR 1242 from adulterated rat feed was made. It became necessary to remove the lipid content (at approximately 7–8%) and this was accomplished via base saponification. We attempted to isolate the AR 1242 from this base saponified matrix and the three chromatograms shown in Figure 3.28G reveal what success we had using SPME and LLE sample prep techniques. The top chromatogram reveals no recovery of AR 1242 in this base-saponified matrix despite adding salt. The middle chromatogram reveals again no recovery of AR 1242 from a portion of the matrix that was spiked with AR 1242 as shown. However, a LLE of the base-saponified matrix recovered AR 1242 as shown! This observation is difficult to explain because

PCBs are neutral and an alkaline matrix should not play such a significant role. Perhaps this is a worthwhile research SPME problem!

We leave the discussion of organics sample prep and move on to inorganics sample prep.

72. HOW ARE THE METHODS CATEGORIZED FOR TRACE INORGANIC ANALYSIS?

A recent compilation of EPA methods organizes the numerous analytical methods for inorganics according to the following six major categories (88):

- Trace metals identified by flame (FIAA) and graphite furnace (GFAA) atomic absorption spectrophotometry
- Trace metals identified by inductively coupled plasma–atomic emission spectrophotometry (ICP–AES)
- Trace metals identified by inductively coupled plasma – mass spectrometry (ICP–MS)
- Mercury identified by cold vapor atomic absorption
- Cyanide (total and amenable)
- Organic carbon (total)

Not listed in these categories are analytical methods for the principal inorganic anions derived from strong acids that are prevalent in ground-water: chloride, bromide, nitrite, nitrate, phosphate, and sulfate. These analytes are currently measured routinely by the application of either ion chromatography (IC) or specific colimetric procedures following the conversion of the anion to a colored complex. Oxyhalides such as the bromate ion have been found in chlorinated drinking water. IC methods have been developed for various oxyhalide ions in recent years. Water that is free of dissolved organics and heavy metals can be directly injected into ion chromatographs or filtered if particulates are present. Aqueous samples that contain dissolved biomatter and/or heavy metal ions pose severe challenges to IC because the columns employed in the technique are susceptible to column fouling.

In this chapter, we will discuss the basis of sample preparation for five of the six categories listed above and focus on the determinative step for all six in Chapter 4. Total organic carbon commonly called TOC is a combustion technology in which aqueous samples can be injected directly without the need for sample preparation; therefore, we will not discuss it any further in this chapter. Let us start with a discussion of the principles of sample prep with respect to trace metals.

73. HOW DO YOU PREPARE AN ENVIRONMENTAL SAMPLE TO MEASURE TRACE METALS?

Sample preparation for the determination of trace concentration levels of the many priority pollutant metals is strongly connected to the nature of the determinative technique. Historically, FIAA was first used to measure metals. The more sensitive GFAA technique followed. Along about the same time as GFAA was being developed, ICP-AES came along. ICP-AES afforded the opportunity to measure more than one metal in a sample at a time, the so-called multielement approach. In recent years, the development of ICP-MS has carried trace metal analysis to significantly lower IDLs and introduced the opportunity to identify and to quantitate the various elemental isotopes.

The metals of greatest interest to TEQA are listed in Table 3.15 along with the author's comment on the chemical and toxicological nature of each metal. The design of instrumentation for either FIAA, GFAA, ICP-AES, or ICP-MS requires that the sample be introduced as a liquid. These systems easily accommodate aqueous solutions in contrast to gas chromatographs that require, for most of the polysiloxane capillary columns, the injection of a nonaqueous liquid such as an organic solvent. Aqueous samples that contain inorganics, in contrast to aqueous samples that contain organics, can be introduced into a FIAA, GFAA, or ICP without removal of the analyte from its sample matrix. Chemically, metals might exist in the environment either as ions in one or more oxidation states or partially or wholly chelated to ligands of various sorts. They may be bound or complexed to soil/sediment particulates and therefore cannot be easily released via a liquid-solid extraction or leaching. Some metals form the structural composition of solid matrices derived from the environment, such as alumino silicates in clay. In these cases, decomposition of the sample removes the organic portion of the matrix. Decomposition methods include the following:

1. Combustion with oxygen with and without fluxes
2. Digestion with acids

Both methods for decomposing the sample matrix require heat and both have benefited from replacement of resistive heating (e.g., laboratory hot-plate equipment) with microwave heating (89).

The following procedure has been used by this author to prepare a sample of fly ash for determination of priority pollutant metals (90): Assume that the ash has been obtained from a previous combustion procedure.

TABLE 3.15 Toxicity, Common Oxidation States, and Chemical Forms of the Most Environmentally Significant Chemical Elements

Element	Toxicity				# Oxidation states	Common chemical forms
	A	B	C	D		
Mg		x			1	Mg(2+)
Al			x		1	Al(3+)
Cr	x				9	Cr(3+),Cr(VI)
Mn		x			11	Mn(IV),Mn(VII)
Fe		x			9	Fe(2+),Fe(3+)
Co		x			7	Co(2+)
Ni	x				7	Ni(2+)
Cu		x			6	Cu(1), Cu(2+)
Zn		x			2	Zn(2+)
Ga			x		3	Ga(III)
As	x				3	As(III),As(V)
Se		x			6	Se(IV),Se(VI)
Mo		x			8	Mo(VI)
Ag				x	4	Ag(+)
Cd	x				2	Cd(2+)
In				x	3	In(III)
Sn				x	2	Sn(II),Sn(IV)
Sb				x	3	Sb(III),Sb(V)
Te				x	7	Te(IV)
Ba				x	1	Ba(2+)
Pt			x		5	Pt(IV)
Au			x		6	Au(III)
Hg	x				2	Hg(+),Hg(2+)
Tl				x	2	Tl(+)
Pb	x				2	Pb(2+),Pb(IV)
Bi			x		4	Bi(III)

Note: Toxicity Categories Explained

A = major toxic metals with multiple effects

B = essential metals with potential for toxicity

C = metals with toxicity related to medical therapy

D = minor toxic metals

Source: Data from CD Klasser, ed. Casarett & Douli's Toxicology: The Basic Source of Poisons, 5th ed. New York: McGraw-Hill, 1996; and J. Elmsley. The Elements. Oxford: Oxford University Press, 1989.

1. Weigh 0.2 g of sample into a 100-mL beaker. Record the weight to the nearest 0.001 g using an analytical balance.
2. Add 5 mL of concentrated nitric acid (conc. HNO_3) and 5 mL of concentrated hydrochloric acid (conc. HCl).
3. Place a watch-glass over the beaker and digest at medium heat for 60 min.
4. Evaporate to dryness.
5. Add 5 mL of conc. HNO_3 and evaporate to dryness.
6. Add 1 mL of conc. HNO_3 and warm.
7. Add 1 mL of distilled deionized water and warm. Filter into a 25-mL volumetric flask.
8. Cool and dilute to the mark using 1% HNO_3 . This gives exactly 25 mL of an aqueous sample containing the solubilized metal ion.
9. Aspirate into a previously calibrated FIAA and record the absorbance.

Notes

1. Be sure to choose the appropriate wavelength and oxidizer gas for each metal of interest. The aqueous solutions used as calibration standards should be prepared in 1% HNO_3 .
2. For every batch of samples that have been digested, one blank, one matrix spike, and one matrix spike duplicate should also be prepared. For the matrix spike and matrix duplicate, designate one sample from the batch for these.

Sample matrices from a biological origin such as blood, urine, and serum can be merely diluted with water, dilute nitric acid, or a dilute surfactant such as Triton X-100 and aspirated directly into the flame for FIAA or placed directly into the graphite tube for GFAA. With respect to GFAA, a plethora of “matrix modifiers” have been developed over the years to deal with spectral and chemical types of interference (91). Spectral interferences arise when the absorption or emission of an interfering species either overlaps or lies so close to the analyte absorption or emission that resolution by the monochromator becomes impossible. Chemical interferences result from various chemical processes occurring during atomization that alter the absorption characteristics of the analyte (92). Spectral interferences refer to the presence of concomitants that affect the quantity of the source light that reaches the detection system, whereas chemical interferences reduce the analyte absorbance signal by interactions with one or more concomitants compared to standards without concomitants (93).

74. WHAT IS MATRIX MODIFICATION IN GFAA?

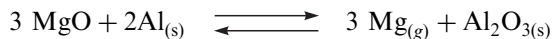
Because matrix modification is one aspect of sample preparation, even though it is most often accomplished automatically in GFAA, we will discuss it here. For example, NIOSH Method 7105 recommends a matrix modifier that consists of a mixture of ammonium dihydrogen phosphate, magnesium nitrate, and nitric acid for the determination of airborne Pb. Because the graphite furnace can be viewed as a chemical reactor whereby the sample with its matrix is placed on a graphite platform (the L'vov platform, discussed in Chapter 4) and heated to a very high temperature, reactions can take place that involve both the metal analyte of interest as well as sample matrix components.

The concept of matrix modification, from the sample prep perspective, is to add to the sample a chemical reagent that will cause a desirable chemical reaction or inhibit an undesirable reaction (94). For metals that tend to volatilize, one can add a modifier that reduces analyte volatility by increasing the volatility of the matrix. Consider the determination of Pb in highly salted aqueous samples such as seawater. Seawater contains appreciably elevated levels of chloride salts. Adding an ammonium ion to the seawater followed by heating the sample to a high temperature causes the following reaction to occur:



This reaction removes chloride ions from the sample while minimizing the loss of Pb as the more volatile PbCl_2 .

Harris discusses the findings of Styris and Redfield, who studied the effect of magnesium nitrate on the determination of Al (95,96). At high temperature, MgO is formed and steadily evaporates. This maintains a steady vapor pressure of MgO in the GFAA tube. The presence of MgO serves to keep Al as the oxide by establishing the following equilibrium:



When most of the MgO has evaporated, the equilibrium begins to shift to the left as Al_2O_3 is converted to elemental Al. This reaction serves to delay the Al from evaporating until a higher temperature is reached.

Butcher and Sneddon describe the work of Schlemmer and Welz, who investigated the use of Pd and Mg nitrates as matrix modifiers in the determination of nine metallic elements (97,98). They showed that higher pyrolysis temperatures could be used as compared to no modifier or using other

common modifiers. Thus, we have thus shown how additions to the sample matrix led to improved performance in GFAA.

With respect to FIAA and ICP, the addition of so-called matrix modifiers developed for GFAA serves no useful purpose. FIAA and ICP use nebulization into an oxidizing–reducing high-temperature source to introduce a liquid into the flame and plasma, respectively, and because of this, both techniques require that samples have a low dissolved solids content so as to prevent clogging. ICP is essentially free from most spectral and chemical interferences due to the extremely high temperature of the plasma (8000–10,000°C), whereas these interferences are prevalent in FIAA and serve to influence the IDL for a given metal.

75. HOW DO I PREPARE A SOLID WASTE, SLUDGE, SEDIMENT, OR SOIL SAMPLE?

EPA Method 3050 from the SW-846 series of methods involves solubilizing a solid sample with acids and peroxide and removing the insoluble residue by filtration. EPA Method 3051 is a microwave-assisted acid-digestion procedure. EPA Method 200.3 is applicable to the preparation of biological tissue samples prior to using atomic spectrometry for quantifications of Al, Sb, As, Ba, Be, Cd, Ca, Cr, Co, Cu, Fe, Pb, Li, Mg, Mn, Hg, Mo, Ni, P, K, Se, Ag, Na, Sr, Tl, Th, U, V, and Zn and an outline of the sample prep procedure is as follows (99):

- Place up to a 5-g subsample of frozen tissue into a 125-mL Erlenmeyer flask. Any sample-spiking solutions should be added at this time and allowed to be in contact with the sample prior to the addition of acid.
- Add 10 mL concentration nitric acid and warm on a hot plate until the tissue is solubilized. Gentle swirling of the sample will aid in this process.
- Increase the temperature to near boiling until the solution begins to turn brown. Cool sample, add an additional 5 mL of concentrated nitric acid, and return to the hot plate until the solution once again begins to turn brown.
- Cool sample, add an additional 2 mL of concentrated nitric acid, return to the hot plate, and reduce the volume to 5–10 mL. Cool sample, add 2 mL of 30% hydrogen peroxide, return sample to the hot plate, and reduce the volume to 5–10 mL.
- Repeat the previous step until the solution is clear or until a total of 10 mL of peroxide has been added.

- Cool the sample, add 2 mL of concentrated hydrochloric acid, return to the hot plate, and reduce the volume to 5 mL.
- Allow the sample to cool and quantitatively transfer to a 100-mL volumetric flask. Dilute with DDI, mix, and allow any insoluble material to separate. The sample is now ready for either ICP–AES or GFAA.

So far, we have considered destroying or transforming the sample matrix in some way while leaving the metal ion intact. We now consider ways to transfer the metal ion from the sample matrix and this leads to sample prep techniques that preconcentrate the sample.

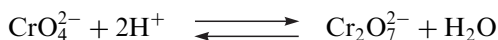
76. WHAT CAN I DO TO PRECONCENTRATE A SAMPLE FOR TRACE METAL ANALYSIS?

There is a need for methods that can detect ultratrace concentration levels of the priority pollutant metal ions that are present in the environment. It may be desirable to detect and measure the concentration of one or more metals from a matrix that is highly salted and it is expected that the concentration of the metals of interest are extremely low [e.g., at parts per trillion (ppt) levels]. An aqueous sample can be preconcentrated by evaporating off the water, precipitating the analyte, followed by redissolution of the precipitate, extracting the analyte via cation exchange or after forming an anionic species from the metal ion, via anion exchange, and isolating the metal ion by first forming the neutral metal chelate and sorbing this chelate on a hydrophobic surface such as a chemically bonded silica or extracting the metal chelate into a nonpolar solvent via LLE. We will focus on three aspects of preconcentration by considering the following examples:

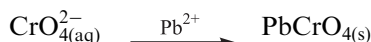
1. The coprecipitation of Cr(VI) using lead sulfate
2. The mathematics for the general case of LLE involving metal ions and the neutral metal chelates that can be formed
3. The mathematics for isolating and recovering a neutral metal chelate, cadmium(II) oxinate, from a spiked aqueous sample.

77. HOW DO I PRECONCENTRATE CR(VI) FROM A LEACHATE USING COPRECIPITATION?

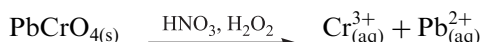
In the environment, Cr(VI) is either in the chromate (CrO_4^{2-}) or dichromate ($\text{Cr}_2\text{O}_7^{2-}$) form. Whether Cr(VI) is predominantly one or the other strongly depends on the pH of the aqueous solution within which it is dissolved. The following ionic equilibrium is established between the two:



Thus, if the aqueous solution that contains CrO_4^{2-} is made acidic by adding a source of the hydronium ion, the equilibrium adjusts to produce more $\text{Cr}_2\text{O}_7^{2-}$. If the hydronium ion is removed by reaction with the hydroxide ion or other base, the equilibrium adjusts to produce more CrO_4^{2-} . Let us assume that we have an aqueous solution containing Cr(VI) as either chromate or dichromate and Cr(III) in the form of CrCl_3 . The addition of a source of the Pb^{2+} ion, such as lead(II) sulfate, will precipitate Cr(VI) as the insoluble PbCrO_4 while leaving Cr(III) in the supernatant as Cr^{3+} . Thus, a speciation of chromium via coprecipitation can be realized. Hence,

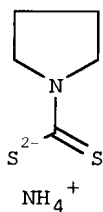


The PbCrO_4 precipitate is washed clean of occluded Cr^{3+} and chemically reduced by the addition of hydrogen peroxide and nitric acid according to



If it is expected that the concentration is within the range of 1–10 mg/L as Cr, the dissolved Cr(III) can now be analyzed after adjustment to a precise volume by either FLAA or ICP–AES. If it is expected that the concentration is within the range of 5–100 $\mu\text{g/L}$ Cr, the Cr(III) could be injected into the GFAA. A procedure for the possible speciation of chromium that might be found in the environment between Cr(III) and Cr(VI) along with the appropriate sample prep is found in EPA Method 7195 from the SW-846 series.

Chromium(VI) can be quantitated without coprecipitation by forming a metal chelate. Method 7196A provides a procedure to prepare the diphenylcarbazone complex with Cr(VI) in an aqueous matrix. The method is not sensitive in that it is useful for a range of concentrations between 0.5 and 50 mg/L Cr. A more sensitive colorimetric method converts Cr(VI) to Cr(VI) chelate with ammonium pyrrolidine dithiocarbamate (APDC), followed by LLE into methyl isobutyl ketone (MIBK). The molecular structure for APDC is as follows:



The APDC forms chelates with some two dozen metal ions (100). The extent of formation of the metal chelate is determined by the magnitude of the formation constant, β . The efficiency of LLE as defined by a metal chelate's percent recovery depends on the distribution ratio, D , of the metal chelate in the two-phase LLE. We use the fundamental definition of D to develop a useful relationship for metal chelate LLE.

78. TO WHAT EXTENT CAN A GIVEN METAL CHELATE BE RECOVERED BY LLE?

Recall the definition of a distribution ratio for a specific chemical species as defined by Eq. (3.9). If a chelate itself is a weak acid, secondary equilibrium plays a dominant role. We start by writing down a definition for the distribution ratio that accounts for all chemical species involving a metal ion M :

$$D = \frac{\sum C_M(\text{organic})}{\sum C_M(\text{aqueous})}$$

This generalization can be reduced to

$$D = \frac{[ML_n]_{\text{organic}}}{[ML_n]_{\text{aqueous}} + [M^{n+}]_{\text{aqueous}}} \quad (3.50)$$

This definition assumes that the only metal-containing species are the neutral chelate, ML_n , and the free metal ion, M^{n+} . This assumption simplifies the mathematics. Other chemical species that might contain the metal are not in appreciable enough concentration to be considered. The degree to which a metal remains as the uncomplexed metal ion, M^{n+} , is given by α_M whereby α_M is the fraction of all of the metal-containing species in the aqueous phase that is in the M^{n+} form (101):

$$\alpha_M = \frac{[M^{n+}]_{\text{aqueous}}}{[ML_n]_{\text{aqueous}} + [M^{n+}]_{\text{aqueous}}} \quad (3.51)$$

Equation (3.51) is solved for the term $\{[ML_n] + [M^{n+}]\}$ and then substituted into Eq. (3.50) to give the following relationship:

$$D = \frac{[ML_n]_{\text{organic}}}{[M^{n+}]} \alpha_M \quad (3.52)$$

Equation (3.52) states that the degree to which a given metal chelate, ML_n , partitions into the organic phase depends on the ratio of the concentration of extracted ML_n to the concentration of free metal ion and on the degree to which metal ion remains uncomplexed in the aqueous phase. Equation (3.52) is quite complex, and as it stands, this equation is not too useful in being able to predict the extraction efficiency. Figure 3.29 is a diagrammatic representation of what happens when a metal ion, M^{n+} , forms a metal chelate with a weak acid-chelating reagent, HL. The metal chelate is formed where one metal ion complexes to n singly charged anionic ligands, L , to form the metal chelate, ML_n . The several equilibria shown set the stage for secondary equilibrium effects in which the concentration of HL and of ML_n , both present in the organic phase, can be changed from a consideration of the effect of pH in the aqueous phase. We now proceed to discuss and derive a much more useful relationship that, once derived, enables some predictions to be made about the LLE extraction efficiency of metal chelates.

79. HOW DO YOU DERIVE A MORE USEFUL RELATIONSHIP FOR METAL CHELATES?

We derive a more useful relationship for the LLE of metal chelates by considering the well-known secondary ionic equilibria described in Figure 3.29. Let us start by assuming that the chelating reagent to be used to complex with our metal ion of environmental interest is, in general, a weak acid. This monoprotic (our assumption) weak acid can ionize only in the aqueous phase and does so according to

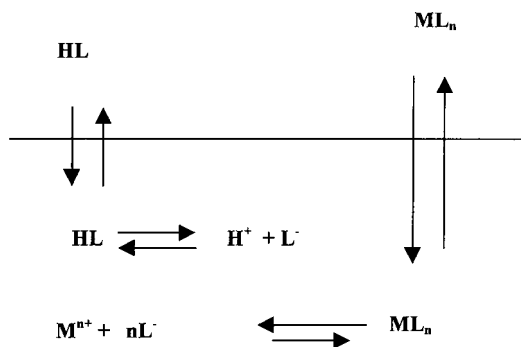
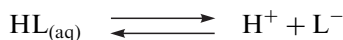


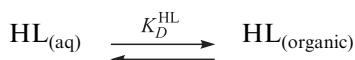
FIGURE 3.29 Various equilibria for the distribution of a metal chelate between two immiscible phases where the chelate itself is a weak acid.



The extent of this dissociation is governed by its acid dissociation constant, K_a , and is defined for the case of a monoprotic weak acid, HL, as follows:

$$K_a = \frac{[\text{H}^+][\text{L}^-]}{[\text{HL}]}$$

The neutral chelate can also partition into the organic phase according to



The extent to which HL partitions into the organic phase is governed by its partition coefficient, K_D^{HL} , and is defined as

$$K_D^{\text{HL}} = \frac{[\text{HL}]_{\text{organic}}}{[\text{HL}]_{\text{aqueous}}} \quad (3.54)$$

Chelating reagents that are amphiprotic such as 8-hydroxyquinoline, HOx, have a more limited range of pH within which the distribution ratio for HOx approximates K_D^{HL} .

Free metal ions, M^{n+} and the conjugate base to the weak acid chelate, L^- , that are present in the aqueous phase will form the metal chelate by reaction of n ligands coordinating around a central metal ion. The extent of complexation is governed by the formation complex, β , according to



The formation constant of the metal chelate in aqueous solution is defined as

$$\beta = \frac{[\text{ML}_n]_{\text{aqueous}}}{[\text{M}^{n+}]_{\text{aqueous}}[\text{L}^-]_{\text{aqueous}}^n} \quad (3.55)$$

The partition coefficient for the neutral metal chelate, ML_n , when a relatively nonpolar and water-immiscible solvent is added to an aqueous solution containing the dissolved metal chelate is given as follows:

$$K_D^{\text{ML}_n} = \frac{[\text{ML}_n]_{\text{organic}}}{[\text{ML}_n]_{\text{aqueous}}} \quad (3.56)$$

The acid-dissociation constant expression, formation constant expression, and the two expressions for the partition coefficients can be substituted into the defining equation for D [Eq. (3.50)], rearranged, and simplified.

80. CAN WE DERIVE A WORKING EXPRESSION FOR THE DISTRIBUTION RATIO?

Yes we can, and we utilize all of the above equations to do so. This is an instructive exercise that can be found in a number of analytical chemistry texts that introduce the topic of metal chelate extraction. Usually the derivation itself is not included and only the final equation is given and interpreted. In the derivation that follows, we find that we do not need to add any more simplifying assumptions to those already given to reach the final working equation.

Let us consider eliminating the concentration of free metal ion by solving Eq. (3.55) for $[M^{n+}]$ and substituting this expression into Eq. (3.52). This gives

$$D = \frac{K_D^{\text{ML}_n} [\text{ML}_n]}{[\text{ML}_n] \beta [\text{L}^-]^n} \alpha_M$$

The concentration of metal chelate in the aqueous phase cancels and we obtain the following expression for D :

$$D = K_D^{\text{ML}_n} \beta [\text{L}^-]^n \alpha_M$$

This equation can be further simplified by eliminating the ligand concentration term by solving Eq. (3.53) for $[\text{L}^-]$ and substituting this expression. We get

$$D = K_D^{\text{ML}_n} \beta \left\{ \frac{K_a [\text{HL}]}{[\text{H}^+]} \right\}^n \alpha_M$$

Rearranging this equation gives

$$D = \frac{K_D^{\text{ML}_n} \beta K_a^n}{[\text{H}^+]^n} [\text{HL}]_{\text{aqueous}}^n \alpha_M$$

This equation can be further simplified by eliminating the concentration of the undissociated weak acid chelate in the aqueous phase by substituting for $[\text{HL}]_{\text{aqueous}}$ using Eq. (3.54). Upon rearrangement, we have the final working relationship for the distribution ratio of the metal chelate ML_n when the chelate itself is a monoprotic weak acid HL:

$$D = \frac{K_D^{\text{ML}_n} \beta K_a^n [\text{HL}]_{\text{organic}}^n}{(K_D^{\text{HL}})^n [\text{H}^+]^n} \alpha_{\text{M}} \quad (3.57)$$

Equation (3.57) is the generalized relationship. This relationship was developed earlier for a specific metal chelate, as was shown in Eq. (3.18). Equation (3.57) is viewed as showing that the magnitude of the distribution ratio depends on the magnitude of the four equilibrium constants. These constants depend on the particular metal chelate. The distribution ratio can be varied by either changing the concentration of chelate in the organic phase or the pH of the aqueous phase. The number of ligands that bond to the central metal ion, n , is also an important parameter. As shown earlier [Eq. (3.16)], once we know D we can calculate E if we know or can measure the phase ratio for LLE. Knowing E enables us to determine the percent recovery and hence to quantitatively estimate the extraction efficiency. Because $100 \times E$ equals the percent recovery for a given metal chelate, a plot of the percent recovery of a given metal chelate versus pH reveals a sigmoid shaped curve. The inflection point in the curve yields the $\text{pH}_{1/2}$ for the specific metal. This is the pH at which 50% of a metal is extracted.

Figure 3.30 is a plot of the percent extracted against the solution pH for four metals, Cu(II), Sn(II), Pb(II), and Zn(II), as their respective dithizonates. The exact value for each metal's $\text{pH}_{1/2}$ is as follows:

Metal dithizone	$\text{pH}_{1/2}$
Cu(II)	1.9
Sn(II)	4.7
Pb(II)	7.4
Zn(II)	8.5

It should become clear that pH is a powerful secondary equilibrium effect that can be used to selectively extract a particular metal from a sample that may contain more than one metal.

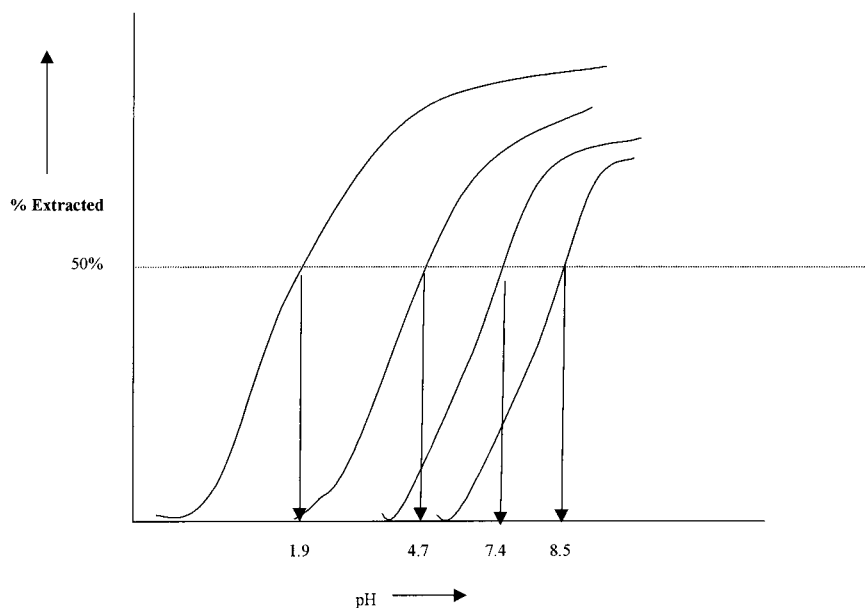


FIGURE 3.30 Plot of the % extracted versus the pH for four methal dithizones.

Day and Underwood have shown that if the logarithm is taken on both sides of Eq. (3.57), we obtain a more useful form (102). Let us first rewrite Eq. (3.57) combining the various equilibrium constants as follows:

$$D = \frac{K_{\text{ex}} [\text{HL}]_{\text{organic}}^n}{[\text{H}^+]_{\text{aqueous}}^n} \quad (3.58)$$

where K_{ex} is substituted for all of the equilibrium constants in Eq. (3.57). Upon taking the logarithm of both sides of Eq. (3.58), we obtain

$$\log D = \log K_{\text{ex}} + n \log [\text{HL}] - n \log [\text{H}^+]$$

This equation can be rewritten in terms of pH as follows:

$$\log D = \log K_{\text{ex}} + n \log [\text{HL}] + np\text{H}$$

A plot of $\log D$ versus pH should in theory be a straight line whose slope is n and whose intercept on the $\log D$ axis (i.e., when $\text{pH} = 0$) is $\{\log K_{\text{ex}} + n \log [\text{HL}]\}$. Figure 3.31 shows such a plot in general terms.

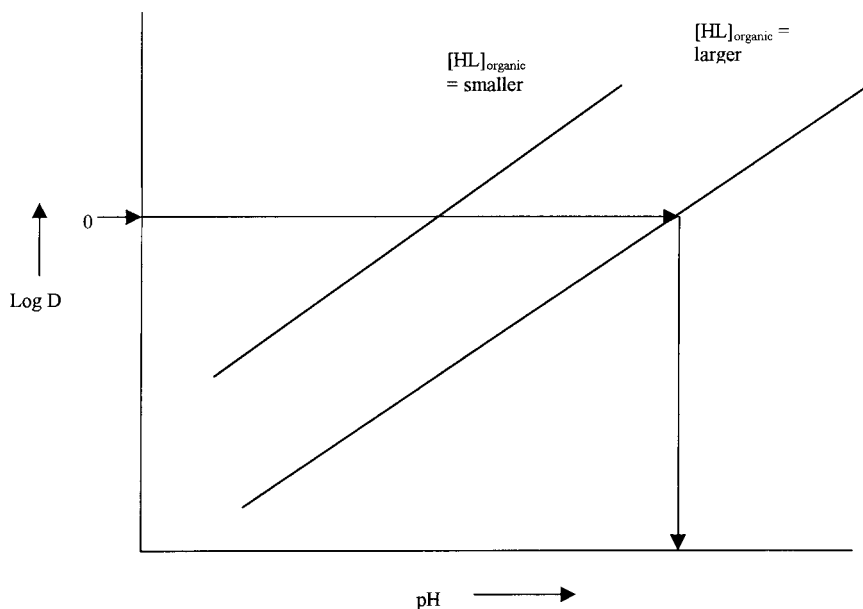


FIGURE 3.31 Plot of the logarithm of the distribution ratio versus pH.

Note that the two lines drawn correspond to two different values for $[\text{HL}]_{\text{organic}}$. These straight lines eventually curve and plateau as the pH of the aqueous phase becomes very high. In this case, the $[\text{H}^+]$ becomes so low that abundant L^- is made available, which, in turn, drives the formation of the metal chelate equilibrium to the right. As more aqueous metal chelate becomes available, the partitioning of the metal chelate shifts in favor of the organic metal chelate. Hence, in the absence of any hydroxide, the value for D approaches, the value for the molecular partition coefficient for the metal chelate, $K_D^{\text{ML}^n}$. Metal hydroxide precipitation at a high pH competes for the free metal ion, M^{n+} , a factor not taken into account during the development of Eq. (3.57).

81. ARE THERE OTHER WAYS TO PRECONCENTRATE METAL IONS FROM ENVIRONMENTAL SAMPLES?

Yes there are, and wouldn't you know it, these are, in essence, SPE-type methods! Both cation- and anion-exchange resins have been used to pre-concentrate inorganic metal cations while removing anionic interferents. A large body of work has been related to the formation of the polychloro

anionic complex such as FeCl_4^- and their isolation using anion-exchange resins.

Chelating resins, styrene-divinylbenzene copolymer, containing iminodiacetate functional groups have been successfully used to preconcentrate transition metal ions from solutions of high salt concentrations. Selective neutral metal chelates have been found to be isolated and recovered using C_{18} chemically bonded silica gel (103). We now digress to some of the author's findings in this area.

82. CAN WE ISOLATE AND RECOVER A NEUTRAL METAL CHELATE FROM AN ENVIRONMENTAL SAMPLE USING BONDED SILICAS?

Neutral metal chelates should behave no differently with respect to the adsorption/partitioning of the species from water to a octadecyl-bonded silica than that of neutral organics, as discussed earlier. This author has developed mathematical equations for the reaction of cadmium ion, Cd^{2+} , with 8-hydroxyquinoline (HOx), also referred to as "oxine," to form a series of complexes. If it is assumed that only the neutral 1:2 complex, CdOx_2 , will partition into the bonded sorbent, equations can be derived that relate the distribution ratio to measurable quantities. We now proceed through this derivation and start with a consideration of the secondary equilibria involved. We first need to consider a more expanded concept, enlarging upon that shown for metal chelate LLE (refer back to Figure 3.25). The schematic in Figure 3.32 depicts the bonded silica-aqueous interface in which only the neutral 1:2 engages in the primary equilibrium, that of partitioning onto or into the monolayer of wetted C_{18} ligates.

The extent to which the free cadmium ion complexes with the oxinate ion to form the 1:1 cation is governed by



The extent to which the cadmium ion complexes with two oxinate anions to form the 1:2 complex is governed by



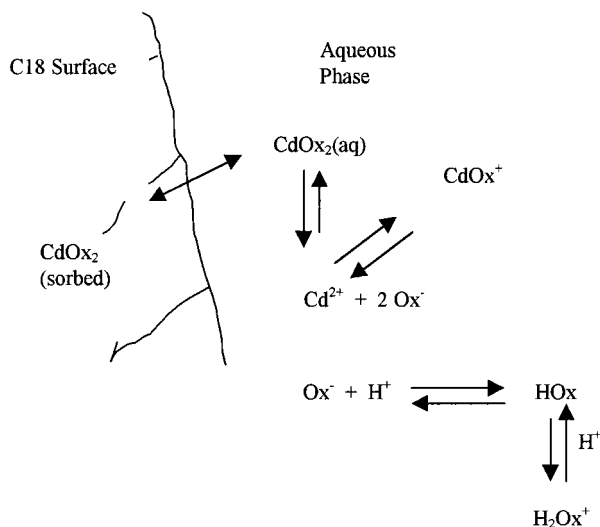
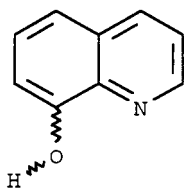


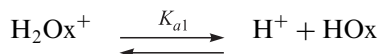
FIGURE 3.32 Various equilibria for the distribution of cadmium oxinate between an aqueous phase and the C₁₈ sorbent surface.

Oxine, itself being amphiprotic, can exist as either the neutral, weak acid or as a protonated species. A molecular structure for oxine as follows:



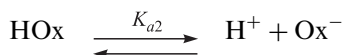
The hydroxyl group behaves as a weak acid, whereas the aromatic nitrogen can accept a proton and behave as a weak base. The degree to which oxine is protonated, H₂Ox⁺, remains neutral, HOx, or dissociates to H⁺ and oxinate, Ox⁻ is governed by the pH of the aqueous phase.

For the protonated species, we can write an equilibrium for the acid dissociation:



The extent of dissociation of this weak acid is governed by the first acid dissociation constant, K_{a2} .

For the neutral weak acid, it too, dissociates in aqueous media according to



The extent to which the neutral form of oxine dissociates is governed by the magnitude of the second acid-dissociation constant, K_{a2} .

If Cd^{2+} and oxine are present in the aqueous phase, these four ionic equilibria would be present to the extent determined by their formation constants. Which complex predominates, the 1 : 1 or 1 : 2? We need to define what we mean by a formation constant for metal complexes and these definitions we take from well established chemical concepts. The formation constant for the 1 : 1 complex is defined

$$\beta_1 = \frac{[\text{CdOx}^+]}{[\text{Cd}^{2+}][\text{Ox}^-]} \quad (3.59)$$

Likewise, the equilibrium expression for the formation of the 1 : 2 complex is given by

$$\beta_2 = \frac{[\text{CdOx}_2]}{[\text{Cd}^{2+}][\text{Ox}^-]^2} \quad (3.60)$$

The acid dissociation constants for the amphiprotic oxine are

$$K_{a1} = \frac{[\text{H}^+][\text{HOx}]}{[\text{H}_2\text{Ox}^+]} \quad (3.61)$$

$$K_{a2} = \frac{[\text{H}^+][\text{Ox}^-]}{[\text{HOx}]} \quad (3.62)$$

Because our discussion centers around trace Cd analysis, let us consider the fraction of all forms of this metal existing as the free, uncomplexed ion δ_0 , where this fraction is defined as

$$\delta_0 = \frac{[\text{Cd}^{2+}]}{C_M} \quad (3.63)$$

Likewise, the fraction of cadmium complexed 1 : 1 is given by

$$\delta_1 = \frac{[\text{CdOx}^+]}{C_M} \quad (3.64)$$

Furthermore, the fraction of cadmium complexed 1 : 2 is given by

$$\delta_2 = \frac{[\text{CdOx}_2]}{C_M} \quad (3.65)$$

We also need to distinguish between the concentration of oxine in the aqueous phase, $[\text{HOx}]$, and the total concentration of oxine, C_{HOx} . This total oxine concentration results from the actual addition of a given amount of the substance to water with adjustment of a final solution volume. With all of these definitions in mind, we can now proceed to substitute and manipulate using simple algebra to help us arrive at more useful relationships than those given by just the definitions.

We start by considering the fraction of cadmium complexed as the 1 : 2 complex. Solving Eq. (3.60) for $[\text{CdOx}_2]$ yields

$$[\text{CdOx}_2] = \beta_2[\text{Cd}^{2+}][\text{Ox}]^2 \quad (3.66)$$

Equation (3.59) can also be solved for the concentration of the 1 : 1 complex:

$$[\text{CdOx}^+] = \beta_1[\text{Cd}^{2+}][\text{Ox}^-] \quad (3.67)$$

Upon substituting Eq. (3.66) into Eq. (3.59) gives

$$\begin{aligned} \delta_2 &= \frac{\beta_2[\text{Cd}^{2+}][\text{Ox}]^2}{[\text{Cd}^{2+}] + [\text{CdOx}^+] + [\text{CdOx}_2]} \\ &= \frac{\beta_2[\text{Ox}^-]^2}{1 + [\text{CdOx}^+][\text{Cd}^{2+}] + [\text{CdOx}_2][\text{Cd}^{2+}]} \end{aligned}$$

Upon substituting Eqs. (3.66) and (3.67) into the denominator in the above expression, we get the following simplified result:

$$\delta_2 = \frac{\beta_2[\text{Ox}^-]^2}{1 + \beta_1[\text{Ox}^-] + \beta_2[\text{Ox}]^2} \quad (3.68)$$

Equation (3.68) is important, but this equation is not the ultimate objective of this derivation! Equation (3.68) states that the fraction of all cadmium can be found in the aqueous phase from a knowledge of only the

two formation constants and the free oxinate ion concentration. It is difficult analytically to measure this free $[\text{Ox}^-]$. There is a solution to this dilemma. Let us consider how the chelate, oxine, is distributed in the aqueous phase. We start by writing a mass balance expression for the total concentration of oxine, C_{HOx} , as follows:

$$C_{\text{HOx}} = [\text{H}_2\text{Ox}^+] + [\text{HOx}] + [\text{Ox}^-]$$

We have thus accounted for all forms that the chelate can take. Equations (3.61) and (3.62) can be solved for the undissociated forms and substituted into the mass balance expression to yield

$$\frac{[\text{H}^+][\text{HOx}]}{K_{a1}} + \frac{[\text{H}^+][\text{Ox}][\text{Ox}^-]}{K_{a2}} + [\text{Ox}^-]$$

Upon further rearrangement and simplification,

$$\frac{[\text{H}^+]}{K_{a1}} \left\{ \frac{[\text{Ox}^-][\text{H}^+]}{K_{a2}} \right\} + \frac{[\text{H}^+][\text{Ox}^-]}{K_{a2}} + [\text{Ox}^-]$$

Factoring out $[\text{Ox}]$ and further rearranging leads to a useful expression:

$$[\text{Ox}^-] \left\{ 1 + \frac{[\text{H}^+]}{K_{a2}} + \frac{[\text{H}^+]^2}{K_{a1}K_{a2}} \right\}$$

We now have as a result the total concentration of oxine in the aqueous phase as being equal to the product of the free oxinate ion concentration and a term that is entirely dependent on the magnitude of the two acid-dissociation constants for oxine and the hydrogen ion concentration or pH. Let us define this term collectively as α and define α as

$$\alpha \equiv \left\{ 1 + \frac{[\text{H}^+]}{K_{a2}} + \frac{[\text{H}^+]^2}{K_{a1}K_{a2}} \right\}$$

The total oxine concentration is thus seen as the product of two terms, so that

$$C_{\text{HOx}} = [\text{Ox}^-]\alpha \quad (3.69)$$

Equation (3.69) can be solved for $[\text{Ox}^-]$ and substituted into Eq. (3.68) to yield the important outcome whereby the fraction of cadmium as the 1 : 2

complex can be expressed entirely in terms of known equilibrium constants and the measurable quantities C_{HOx} and α :

$$\delta_2 = \frac{\beta_2 C_{\text{HOx}}^2}{\alpha^2 + \beta_1 C_{\text{HOx}} \alpha + \beta_2 C_{\text{HOx}}^2} \quad (3.70)$$

The fraction of cadmium as the 1 : 1 complex, δ_1 , is defined as

$$\delta_1 = \frac{[\text{CdOx}^+]}{C_{\text{M}}}$$

$$\delta_1 = \frac{\beta[\text{Ox}^-]_1}{1 + \beta[\text{Ox}^-] + \beta_2[\text{Ox}^-]^2}$$

δ_1 can be expressed in terms of the concentration of free oxinate and is shown as follows. As we did for the 1 : 2 complex, δ_1 can be expressed in terms of measurable quantities as follows:

$$\delta_1 = \frac{\beta_1 C_{\text{HOx}}}{1 + \beta_1 C_{\text{HOx}} + \beta_2 C_{\text{HOx}}^2 / \alpha} \quad (3.71)$$

Finally, the fraction of cadmium as the free ion, δ_0 , is

$$\delta_0 = \frac{[\text{Cd}^{2+}]}{C_{\text{M}}}$$

δ_0 can be expressed as well in terms of the free oxinate concentration:

$$\delta_0 = \frac{1}{1 + \beta_1[\text{Ox}] + \beta_2[\text{Ox}^-]^2}$$

δ_0 can be expressed in measurable quantities and is shown as

$$\delta_0 = \frac{1}{1 + \beta_1 \left\{ \frac{C_{\text{HOx}}}{\alpha} \right\} + \beta_2 \left\{ \frac{C_{\text{HOx}}}{\alpha^2} \right\}^2} \quad (3.72)$$

83. HOW DOES THE FRACTION OF CADMIUM FREE OR COMPLEXED VARY?

Equations (3.70)–(3.72) show that the fraction of cadmium as the 1 : 2 complex, 1 : 1 complex, and the free cation depend entirely on the magnitude of β_1 , β_2 , the concentration of total oxine, C_{HOX} , and pH. This is a significant finding! Using the following values for the respective equilibrium constants, the fraction δ_0 is plotted against pH for a given value for C_{HOX} . Likewise, δ_1 and δ_2 can be plotted against pH on the same graph to yield three distribution curves (104):

$$K_{a1} = 9.84 \times 10^{-6}, \quad \beta_1 = 1.59 \times 10^7$$

$$K_{a2} = 1.23 \times 10^{-10}, \quad \beta_2 = 12.56 \times 10^{13}$$

Figure 3.33 shows how the fraction of free cadmium ion, δ_0 , the fraction of 1 : 1 cadmium oxinate, δ_1 , and the fraction of 1 : 2 cadmium oxinate, δ_2 , vary with a change in aqueous phase pH for a C_{HOX} of 0.5 ppm. As the pH of the aqueous phase is increased, most of the cadmium starts out as Cd^{2+} but gradually is complexed as the 1 : 1 and 1 : 2 complex. The fraction of total cadmium existing as the 1 : 1 complex reaches a maximum at around 8.8, then decreases, whereas the fraction as the 1 : 2 complex continuously increases until all of the metal is complexed as the 1 : 2 complex at around

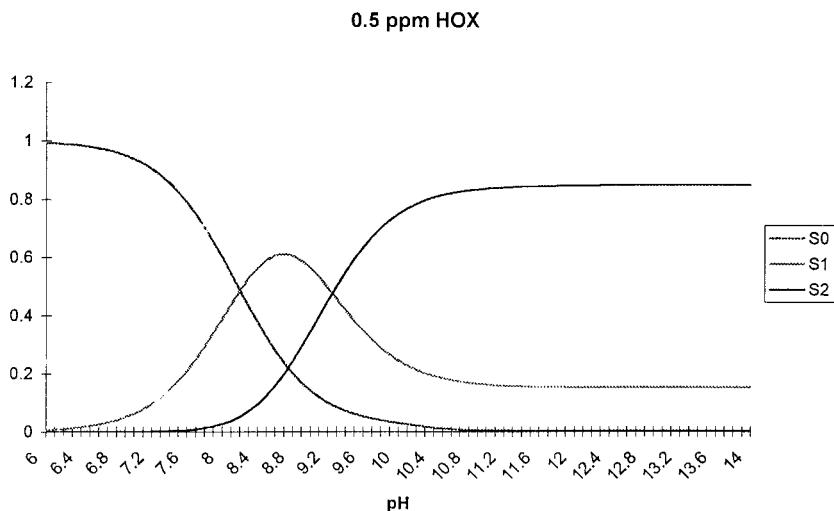


FIGURE 3.33 Distribution diagram for the fraction of free, 1 : 1, and 1 : 2 cadmium oxinates versus pH for a fixed concentration of oxine at 0.5 ppm.

pH = 10. Figure 3.34 is a similar plot but this time for a C_{HOx} of 5.0 ppm. Note that the pH in which the 1 : 1 and 1 : 2 complexes are formed is lower than that for the lower value of C_{HOx} . Figure 3.35 is a similar plot except that C_{HOx} is 50 ppm. The pH at which the complexes are formed is further shifted to lower values. Figure 3.36 is a similar plot except that C_{HOx} is 400 ppm. At this higher total oxine concentration, the pH at which the complexes form is shifted over one pH unit lower versus the case for a C_{HOx} of 50 ppm (105)! A knowledge of the various δ values enables a relationship to be developed for the distribution ratio.

84. CAN AN EQUATION BE DERIVED USING THESE δ VALUES TO FIND D?

The distribution ratio for the partitioning of cadmium oxinate 1 : 2 complex from an aqueous phase to a C_{18} bonded surface can be defined in terms of a ratio of the concentration of CdOx_2 that would be present on the surface to the sum of all of the forms of the metal in the aqueous phase. This is defined as

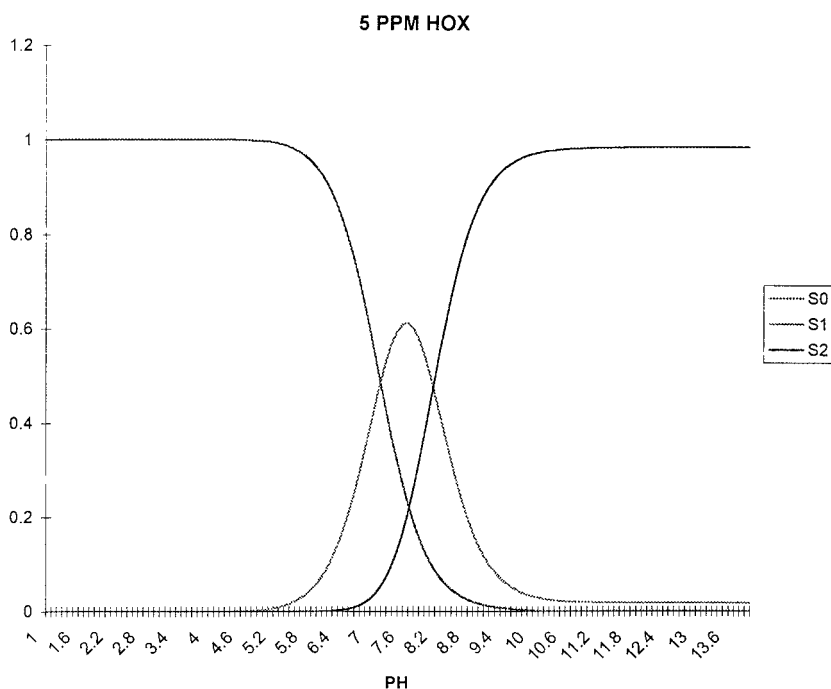


FIGURE 3.34 Distribution diagram for the fraction of free, 1 : 1, and 1 : 2 cadmium oxinates versus pH for a fixed concentration of oxine at 5 ppm.

50 ppm HOX

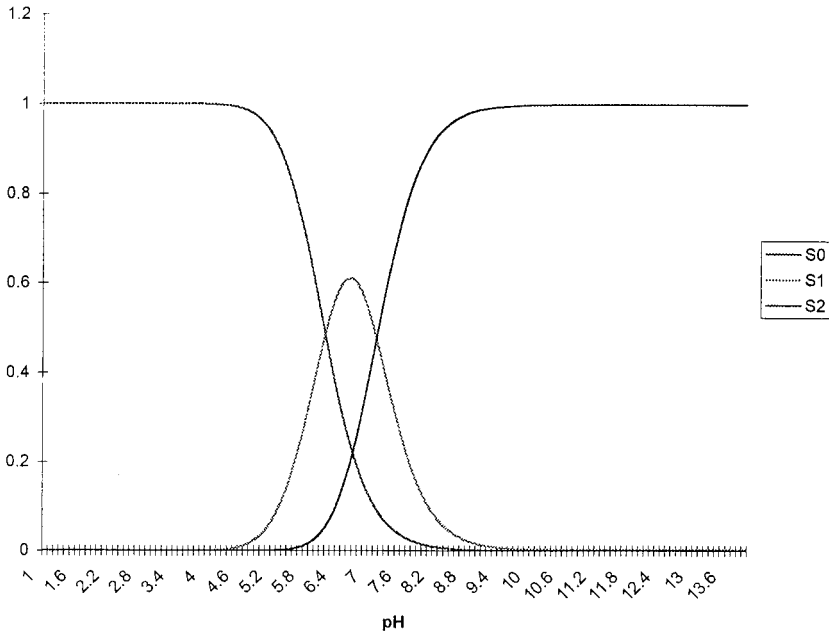


FIGURE 3.35 Distribution of diagram for the fraction of free, 1 : 1, and 1 : 2 cadmium oxinates versus pH for a fixed concentration of oxine at 50 ppm.

$$D_{\text{CdOx}_2} \equiv \frac{[\text{CdOx}_2]_{\text{surface}}}{[\text{Cd}^{2+}] + [\text{CdOx}^+] + [\text{CdOx}_2]} \quad (3.73)$$

and the partitioning coefficient for the molecular form of the species can be defined as

$$K_D^{\text{CdOx}_2} = \frac{[\text{CdOx}_2]_{\text{surface}}}{[\text{CdOx}_2]_{\text{aqueous}}} \quad (3.74)$$

Given

$$\begin{aligned} [\text{Cd}^{2+}] &= \delta_0 C_M \\ [\text{CdOx}^+] &= \delta_1 C_M \\ [\text{CdOx}_2] &= \delta_2 C_M \end{aligned}$$

Upon eliminating these bracketed concentrations in Eq. (3.74) leads to

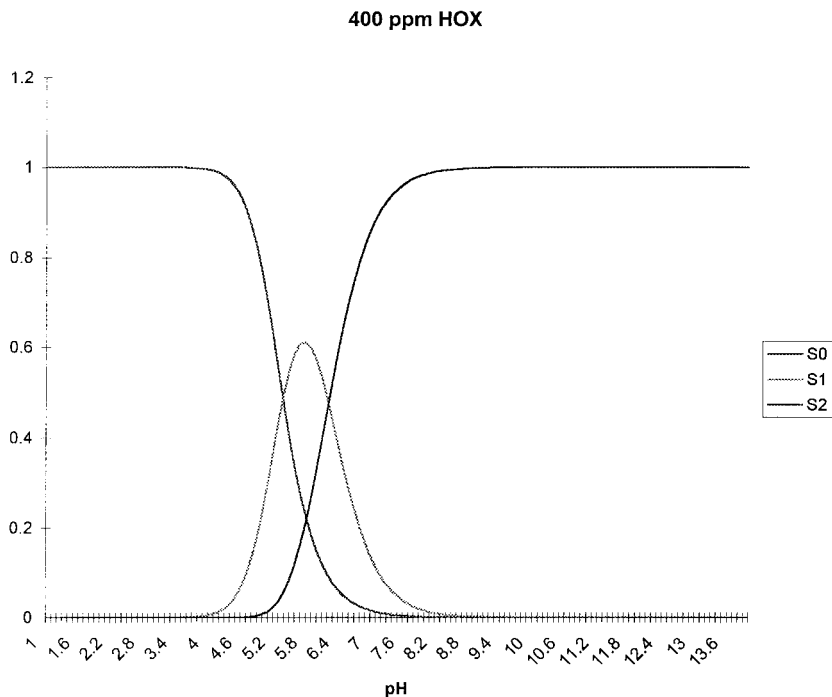


FIGURE 3.36 Distribution diagram for the fraction of free, 1 : 1, and 1 : 2 cadmium oxinates versus pH for a fixed concentration of oxine at 400 ppm.

$$D_{\text{CdOx}_2} = \frac{K_D^{\text{CdOx}_2} \delta_2 C_M}{\delta_0 C_M + \delta_1 C_M + \delta_2 C_M}$$

Upon simplifying and rearranging, we get an important result:

$$D^{\text{CdOx}_2} = K_D^{\text{CdOx}_2} \left\{ \frac{\delta_2}{\delta_0 + \delta_1 + \delta_2} \right\} \quad (3.75)$$

Equation (3.75) is an important outcome of our derivation efforts. Let us proceed to interpret this equation as it relates to the cadmium–oxine system. Equation (3.75) states that the distribution ratio for the 1 : 2 complex between an aqueous phase and a chemically bonded silica such as a C_{18} sorbent depends on the magnitude of the molecular partition coefficient and the degree to which cadmium is found as the 1 : 2 complex. Given that Eqs. (3.70)–(3.72) enable one to calculate δ_0 , δ_1 , and δ_2 , respectively, and that we

have already established that these fractions depend only on C_{HOx} and the pH, we need only to know the pH of the aqueous phase, the molecular partition coefficient from independent studies, and the total oxine concentration in the aqueous phase to calculate D . These parameters were incorporated into an EXCEL spreadsheet to calculate D for various values of pH at different C_{HOx} . Figure 3.37 is a plot of $\log D$ against pH for $C_{\text{HOx}} = 0.5$ ppm; Figure 3.38 is similar but for $C_{\text{HOx}} = 5$ ppm and in a similar manner for Figure 3.39 for $C_{\text{HOx}} = 50$ ppm. Referring to Figure 3.37, we observe that the curve is linear between a pH of around 6.4 up to approximately 8.8 and then the curve levels off, hence becoming independent of pH from about a pH of 9.2 all the way to 14. Figures 3.38 and 3.39 look similar except where on the pH scale the curve crosses. This crossing corresponds to a $\log D = 0$.

Let us examine this a bit further. Taking the logarithm of both sides of Eq. (3.75) gives the following:

$$\log D_{\text{CdOx}_2} = \log K_D^{\text{CdOx}_2} + \log \frac{\delta_2}{\delta_0 + \delta_1 + \delta_2}$$

Where the curve crosses the pH axis, $\log D = 0$ so that

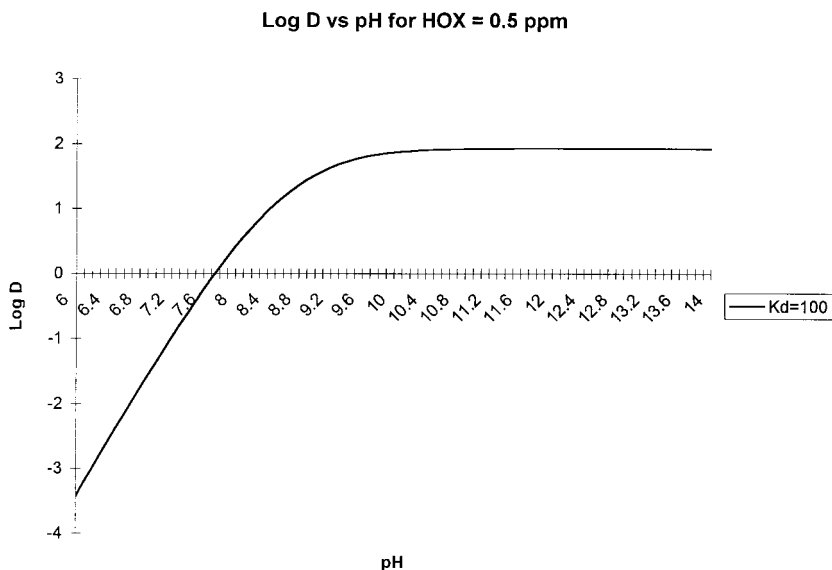


FIGURE 3.37 Plot of the logarithm of the distribution ratio for the 1 : 2 cadmium oxinates versus pH for a fixed concentration of oxine at 0.5 ppm.

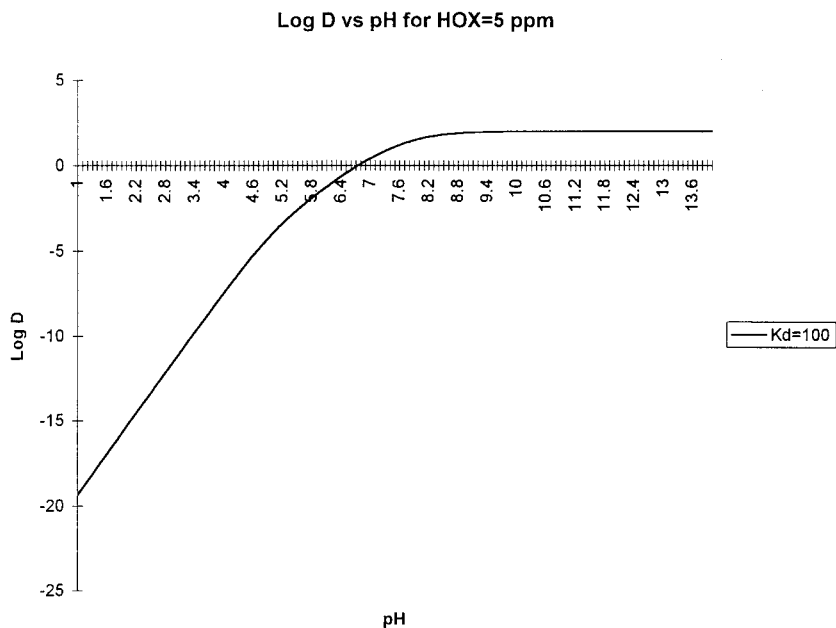


FIGURE 3.38 Plot of the logarithm of the distribution ratio for the 1 : 2 cadmium oxinate versus pH at a fixed concentration of oxine at 5 ppm.

$$0 = \log K_D^{\text{CdOx}_2} + \log \frac{\delta_2}{1}$$

Hence, at the pH where the curve crosses,

$$-\log K_D^{\text{CdOx}_2} = \log \delta_2$$

We have assumed a value for $K_D = 100$ so that $\delta_2 = 0.01$. The curve crosses the pH axis at which the fraction of 1 : 2 complex formed is 0.01 and the percent of 1 : 2 complex is 1%. The pH at which the δ_2 curve just begins to appear, in, for example, Figure 3.33, is about 7.8. This is the same pH value at which the curve in Figure 3.37 crosses the pH axis!

85. HOW GOOD IS THE PREDICTION OF EQ. (3.75)?

We now discuss the results of studies that show the strong dependence of D on the pH of the aqueous phase. We first, however, introduce the experimental procedure used to generate the data. To about 50 mL of distilled

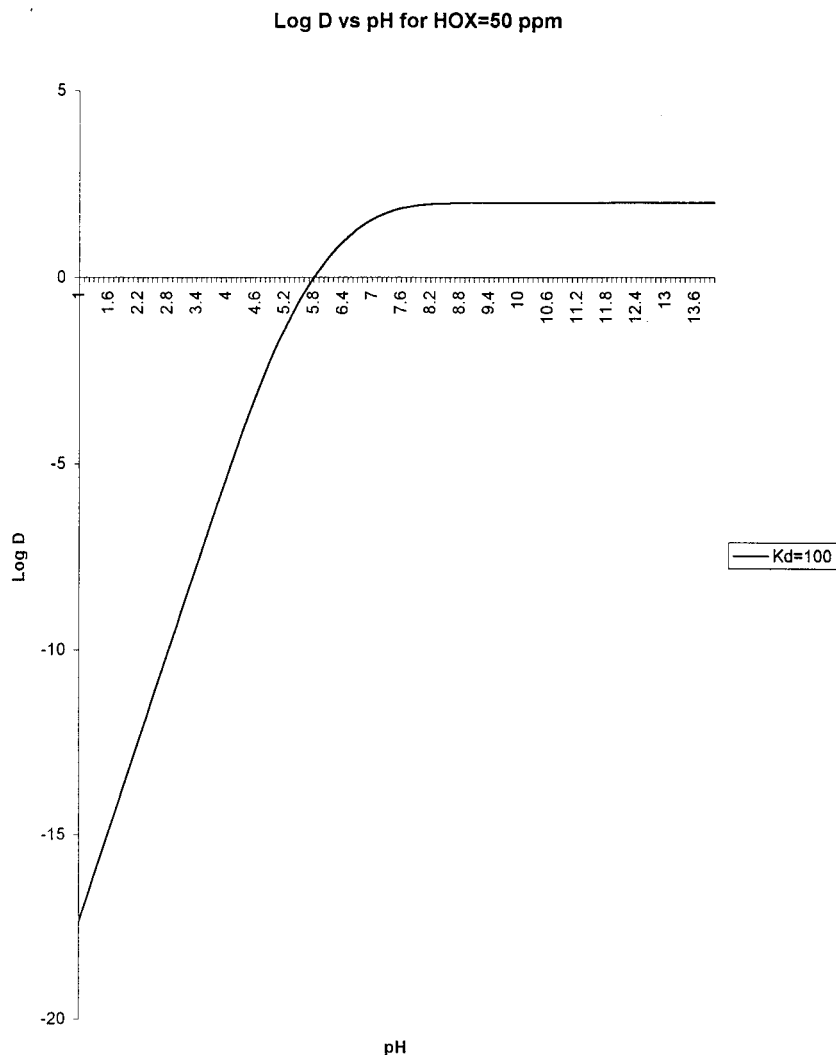


FIGURE 3.39 Plot of the logarithm of the distribution ratio for the 1 : 2 cadmium oxinate versus pH at a fixed concentration of oxine at 50 ppm.

deionized water (DDI) is added an aliquot of a 1% solution of oxine dissolved in methanol. An aliquot of a cadmium salt solution is then added. The pH is adjusted to the desired value with 1% ammonia. This spiked sample is passed through a conditioned C_{18} -bonded sorbent and a brightly colored band is seen near the top of the column. The SPE cartridge is

subsequently eluted with 5 mL MeOH directly into a 10-mL volumetric flask used as a receiver. The contents of the volumetric flask are brought to the calibration mark with 1% nitric acid in DDI. This is a high-purity acid deemed suitable for atomic absorption spectrophotometric analysis. The contents in the volumetric flask are aspirated into a Model 303 Flame AA (Perkin-Elmer). The metal, cadmium, is quantitated based on an external standard mode of instrument calibration in 50 : 50 MeOH : 1% HNO₃. Ten replicate SPEs were conducted at pH 6.2 and at 8.8. The absorbance was measured and the data are shown in Table 3.16 (105). It is evident that at (105) a pH of 6.2, little to no cadmium was recovered, whereas at pH 8.8, almost 30 times as much cadmium was recovered! Two other studies on the isolation and recovery of Cd²⁺ by chelating the ion with oxine and isolating the complex on C₁₈ and C₈ chemically bonded silica gave similar results.

We end this chapter with a brief discussion of two sample preparation methods. The first is to determine mercury (Hg) and the second method determines cyanide (CN⁻). Both parameters are important with respect to the evaluation of a sample as a hazardous waste. The presence of either Hg or cyanide renders a sample hazardous due to the acute toxicity of both species. Mercury exists either in elemental form, as the dimer ion Hg₂²⁺ in which the oxidation state of the element is +1, or as the divalent ion Hg²⁺. The elemental form is the familiar liquid silver metal obtained by roasting cinnabar, HgS (106). The cyanide ion is a moderately strong base derived from the weak acid hydrocyanic acid, HCN. HCN is a gas at room tem-

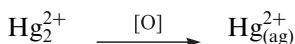
TABLE 3.16 Measured Absorbance for Recovered Cadmium as the Oxine Complex at an Acidic and an Alkaline pH

SPE cartridge #	Weight (mg)	A at pH 6.2	A at pH 8.8
1	199	0.003	0.217
2	202	0.035	0.237
3	272	0.021	0.272
4	310	0.032	0.236
5	415	0.007	0.301
6	415	0.006	—
7	508	0.009	0.290
8	517	0.006	0.290
9	648	0.006	0.348
10	785	0.004	0.215
Mean for <i>n</i> replicates		0.01	0.27
Confidence interval at 95% probability		0.009 (<i>n</i> = 10)	

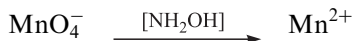
perature and highly toxic. The fear is that hazardous wastes that contain cyanide, if acidified, could release HCN. Cyanide also forms complexes to many metal ions.

86. HOW DO YOU PREPARE A SAMPLE FOR HG DETERMINATION?

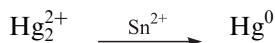
This author has always been fascinated by the oxidation and reduction reactions that Hg undergoes. Because mercury might be present in environmental samples in either of its three oxidation states, an initial and vigorous oxidation would convert all forms of the element to its highest oxidation state, Hg^{2+} . Also, organomercury compounds whereby Hg is in a further reduced state such as dimethylmercury, $(\text{CH}_3)_2\text{Hg}$, also would be oxidized to Hg^{2+} . The common oxidizing agent used is potassium permanganate, KMnO_4 . This is diagrammed as follows:



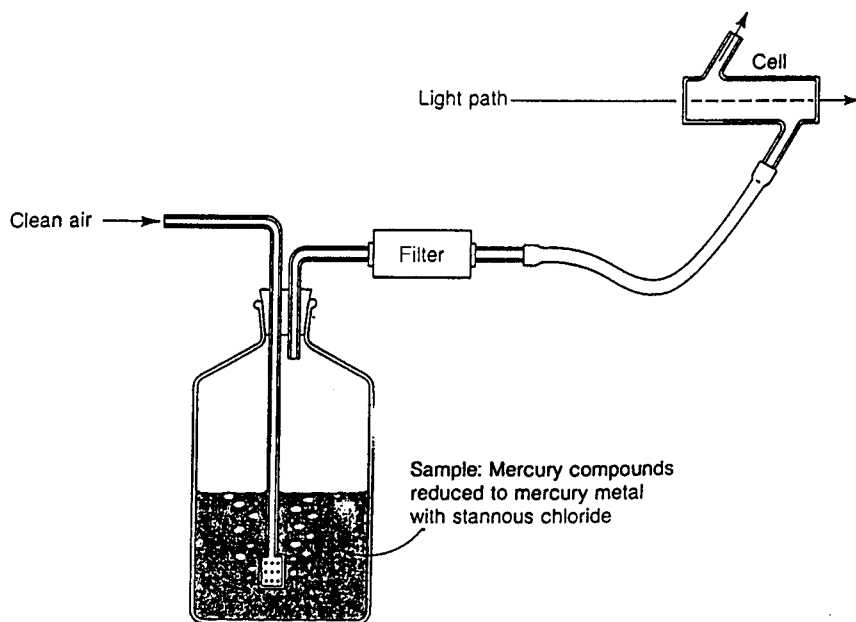
Both EPA Method 7470, applicable to liquid waste, and Method 7471 A, applicable to solid waste, use KMnO_4 . The purple color of the permanganate ion is discharged as this oxidizing agent gets reduced. An excess of MnO_4^- that persists provides visual evidence that oxidation is complete. Further oxidation is afforded by adding potassium persulfate $\text{K}_2\text{S}_2\text{O}_8$. Excess MnO_4^- is reduced using hydroxylamine according to



Tin (II) sulfate is used to reduce all Hg^{2+} to elemental Hg:



The elemental Hg is subsequently purged and swept out of the sample. Figure 3.40 illustrates what accessories are needed to prepare a sample for direct introduction of elemental mercury vapor into a flow-through atomic absorption cell. This modification to the atomic absorption spectrophotometer is called a cold vapor generator.



Flameless atomic absorption analyzer for mercury

FIGURE 3.40 Glass apparatus for sample prep to quantitate elemental mercury.

A typical procedure to determine the concentration of Hg for a drinking water sample is now summarized. This is a routine procedure used in environmental testing labs that perform trace Hg determinations. It reflects EPA Method 245.1 for the determination of Hg in aqueous environmental samples such as surface, saline, and wastewater. A 100-mL sample or equivalent spiked sample is placed into the reaction vessel of the cold vapor generator. A 300-mL BOD (Biological Oxygen Demand) bottle is frequently used for this purpose. To this vessel are added 5 mL of concentrated sulfuric acid, 2.5 mL of concentrated nitric acid, 15 mL of 5% KMnO_4 , and 50 mL of DDI. The contents of the container are mixed well and allowed to stand for 15 min. If the purpose color is discharged during this time, aliquots of 5% KMnO_4 can be added until the purple coloration persists for 15 min. After this, 8 mL of 5% $\text{K}_2\text{S}_2\text{O}_8$ is added to the reaction vessel. The vessel is capped and heated in a 95°C heater block or water bath for 2 h. The contents of the reaction vessel are then cooled and 6 mL of a 12% NaCl/12% hydroxylamine hydrogen sulfate (or alter-

natively hydroxylamine hydrochloride) is added. The reaction vessel is immediately connected to the cold vapor generator and the headspace is purged for 30 s to remove chlorine and other interferences. Next, 5 mL of a 10% tin(II) chloride solution in 0.5N sulfuric acid is injected into the reaction vessel. The Hg vapor is swept into the absorption cell of the atomic absorption (AA) spectrometer where the absorbance at 253.7 nm is measured. Labs generally dedicate one of the AA spectrometers to trace Hg determinations.

Cold vapor generators are but one of the four major atomization devices used in AA, the other three being flames (FIAA), electrothermal (GFAA), and hydride generators. The cold vapor generator shown in Figure 3.40 could also be used as a hydride generator. Hydride generators convert the following elements to their gaseous hydrides: Sb, As, Bi, Se, Te, and Sn. A comparison of method detection limits (MDLs) reveals the real advantage of hydride generator. For example, a MDL for direct aspiration of selenium into a flame with measurement as atomic Se gives 100 ppm, whereas measurement as the hydride gives an MDL of 0.03 ppm (107).

87. HOW IS AN ENVIRONMENTAL SAMPLE PREPARED TO DETERMINE TRACE CYANIDE?

The tendency of free or bound CN^- to become the toxic gas HCN discussed earlier is exploited in the sample prep approach to quantitatively determining cyanide. A wastewater or solid waste sample is placed in (preferably) a three-hole distillation pot. One hole in the pot contains a distilling head and condenser, the second hold is used to introduce purge gas, and the third hole is used for the addition of acid. Following the addition of acid, the pot is sealed. Purge gas is introduced and cyanide is converted to HCN. The HCN is liberated and trapped in a "scrubber." The scrubber contains a dilute NaOH solution which converts HCN back to the cyanide ion. The contents of the scrubber now consist of NaCN in excess hydroxide. This completes the sample prep. The sample is now subject to whatever determinative technique is used to quantitate cyanide. The glassware (two-hole) setup for cyanide distillation from EPA Method 9010B in the SW-846 series is shown in Figure 3.41. The inlet tube along with the connection to a low-vacuum source is used to provide purge air. As HCN gas is removed from the distilling flask, it is trapped in the gas scrubber.

Simple distillation is used to prepare the wastewater sample for the determination of total phenols. An acidified aqueous sample is merely simple distilled with the aqueous distillate trapped into a scrubber containing dilute NaOH, as was the case for cyanide. The contents of the scrubber

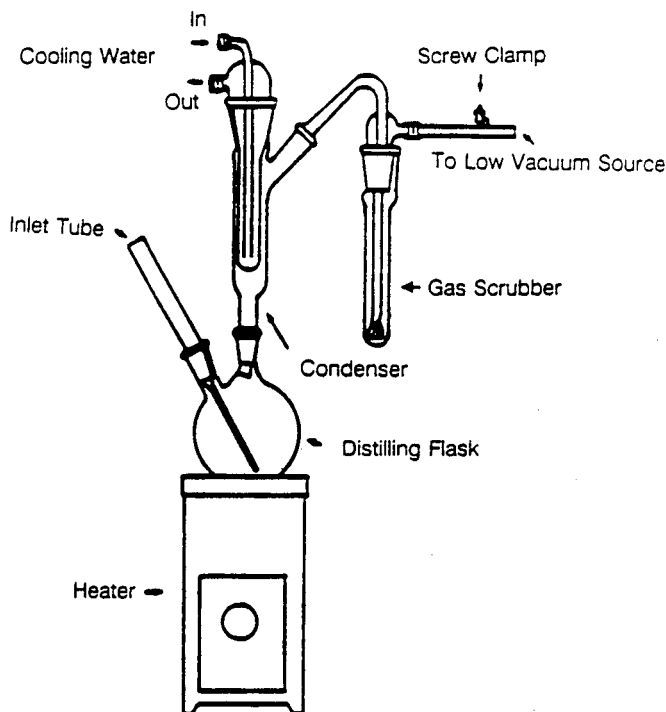


FIGURE 3.41 Glass apparatus for sample prep to quantitate cyanide.

consist of a dilute solution containing sodium phenolate. This solution is subject to whatever determinative technique is applicable to measure trace total phenolics.

88. WHAT CAN WE CONCLUDE ABOUT SAMPLE PREP?

This chapter has introduced the conventional approaches as well as the most recent approaches taken to prepare samples for the determinative techniques that are to follow in Chapter 4. Sample prep was once considered the “bottleneck” with respect to trace organics analysis. This is no longer the case, as has been discussed. The past 15 years have witnessed a revolution in sample prep and this bodes well for significant increases in lab productivity in the future.

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4

Determinative Techniques to Measure Organics and Inorganics

To the observer who does not have the technical background in Trace Environmental Quantitative Analysis (TEQA) and walks into a contemporary environmental testing laboratory, a collection of “black boxes” (instruments) with cables connecting the black boxes to personal computers and other “high-tech” devices should be what makes the first impression. This observer will see people, some of whom wear white lab coats, “running around” holding various glassware such as vials, syringes, beakers, test tubes, or whatever else it is lab people handle when at work in the busy lab. They will, upon being invited to tour, see different departments within the corporate structure. Some department personnel process analytical data generated by these black boxes; some department personnel prepare samples for “introduction” into the black boxes; some department personnel enter data into a laboratory information management system (LIMS) that reads a bar code label on a given sample and tracks the status of that sample as various analytical methods and instruments are used to generate the data. Some instruments are noisy; some are silent; some incorporate robotlike arms; some incorporate samples directly, whereas others require sample preparation; all contribute to the last and no less important step in TEQA—that of determination. Instruments, computers, and accessories all comprise what the Environmental Protection Agency (EPA) refers to as *determinative tech-*

niques, hence the title of this chapter. In Chapter 2, we discussed the important outcomes of using determinative techniques to perform TEQA. In Chapter 3, we discussed the means by which environmental samples are made “suitable and appropriate for introduction to these instruments” (i.e., the science of sample preparation for TEQA). This chapter on determinative techniques therefore completes the thorough discussion of TEQA.

To the sufficiently educated observer, the contemporary environmental testing laboratory is a true testimonial to man’s ingenuity, a “high-tech masterpiece.” However, unlike a work of art, this observer is quick to discover that this artistic endeavor is a “work in progress.” This observer may see a robotic arm of an autosampler depositing 5 μ L of sample into the graphite tube of a graphite furnace atomic absorption spectrophotometer (GFAA). He may also peer into a monitor that reveals an electron-impact mass spectrum of a priority pollutant, semivolatile organic compound. He will become aware very quickly whether or not this particular instrument is running samples or is still running calibration standards in an attempt to meet the stringent requirements of EPA methods. If this person is interested in the progress made by a particular sample as it makes its way through the maze of methods, he can find this information by peering into the sample status section of the LIMS software.

This chapter takes the reader from the uninformed observer described in the first paragraph above to the educated observer who can envision the inner workings of a contemporary environmental testing laboratory. This is the chapter that deals with the “determinative” step, a term coined by EPA. Beginning with their SW-846 series of methods, the sample prep portion was separated from the determinative portion. This separation enabled flexibility in conceptualizing the total method objectives of TEQA in the SW-846 series. This author believes that separating the sample prep from the determinative also makes sense in planning the organization of this book.

A plethora of well-written texts are available on the topic of instrumental analysis. Those that most influenced this author over the years are cited in the references (1–9). Texts are also getting easier to understand and continue to get larger as newer techniques are introduced and already existing instrumentation is continuously improved. The table of contents of such texts vary in topics and in order of presentation. For example, Skoog’s 4th edition text starts with general concepts, then moves to electronics, and then to optical spectroscopy (9). After surveying atomic [atomic absorption (AA) and inductively coupled plasma–atomic emission (ICP–AES)] and molecular spectroscopy, nuclear interactions follow [nuclear magnetic resonance (NMR) and x-ray], and after this, the authors add the topic of mass spectrometry. Electroanalytical chemistry follows and the text proceeds to discuss the separation sciences, namely gas chromatography (GC) and high perfor-

mance liquid chromatography (HPLC), and ends with capillary electrophoresis (CE) and supercritical fluid chromatography (SFC), flow injection, and segmented flow systems.

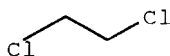
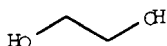
Of the plethora of instrumental techniques, GC, GC–MS (mass spectrometry), HPLC, AA, ICP–AES, and ICP–MS are the principal determinative techniques employed to achieve the objectives of TEQA as applied to both trace organics and trace inorganics analysis. The separation sciences have been coupled to the optical spectroscopic and mass-spectrometric sciences to yield very powerful so-called “hyphenated instruments.” These six techniques are also sensitive enough to give analytical information to the client that is the most relevant to environmental site remediation. For example, one way to clean up a wastewater that is contaminated with polychlorinated volatile organics (ClVOCs) is to purge the wastewater to remove the contaminants, a process known as air stripping. It is important to know that the air-stripped wastewater has a concentration of ClVOCs that meets a regulatory requirement. This requirement is usually at the level of low parts per billion. A determinative technique that can only measure as low as parts per hundred has no place in the arsenal of analytical instruments pertinent to TEQA. Recall from Chapter 2 that techniques relating the acquisition of data directly from analytical instruments provide instrumental detection limits (IDLs), whereas method detection limits (MDLs) combine the sample prep step with the determinative step. This combination serves to significantly lower the overall detection limits and is one of the prime goals of TEQA.

This chapter will focus on fundamentals of instrumental analysis from a viewpoint of using instruments to satisfy the necessary objectives of the determinative step in TEQA. This author hopes not to merely reproduce the many good texts on the subject of instrumental analysis but to add something that is most pertinent to TEQA. We will first discuss the basis of column chromatographic separation that leads to the most important concept (i.e., chromatographic resolution). We will then link together separation science with the means to detect the analyte, a vital ingredient to TEQA. We also need to discuss the hyphenated GC–MS determinative technique that is so important to the contemporary practice of TEQA. We will then leave the separation sciences and discuss the basis for atomic absorption and inductively coupled plasma–atomic emission spectroscopy. We will also discuss ultraviolet–visible (UV-vis) spectrophotometry that continues to play an important role in TEQA. The use of Fourier-transform infrared absorption spectrophotometry to measure oil and grease is so important in TEQA that we must address the scientific basis of this instrumental technique. We also need to discuss the nature of suppressed conductivity detection that forms the basis of ion chromatography, an instrumental technique of huge importance in drinking and other high-

purity water requirements. We will then complete the chapter with a discussion of the fundamentals of inorganic capillary electrophoresis while drawing primarily on this author's work in this field.

1. HOW DO YOU KNOW WHICH DETERMINATIVE TECHNIQUE TO USE?

Which determinative technique to use is dictated by the physical and chemical nature of the analyte of interest. The organics protocol flowchart introduced in Chapter 2 serves as a useful guide. Let us consider how we would determine which instrumental technique to use for the following example. Ethylene glycol, 1,2-ethanediol (EG), and 1,2-dichloroethane (1,2-DCA) consist of molecules that contain a two-carbon backbone with either a hydroxyl- or chlorine-terminal functional group. The molecular structures for these are as follows:



These two molecules look alike; so could not we use the same instrument and conditions to quantitate the presence of both of these compounds in an environmental sample? Nothing could be further from the truth! Some relevant physical properties of both compounds are given in Table 4.1. The presence of two hydroxyl groups enables ethylene glycol to extensively hydrogen-bond both intramolecularly (i.e., to itself) and intermolecularly (i.e., between molecules) when dissolved in polar solvents such as water and methanol. In stark contrast to this associated liquid, 1,2-DCA interacts intramolecularly through much weaker van der Waals forces and is incapable of interacting intermolecularly with polar solvents while being miscible in nonpolar solvents such as chloroform and ether. The boiling point of EG is almost twice as large as that of 1,2-DCA! These significant differences in physical properties would also be reflected in octanol–water partition coefficients. 1,2-DCA can be efficiently partitioned into a nonpolar solvent or to

TABLE 4.1 Physico-chemical Properties of Ethylene Glycol and 1,2-Dichloroethane

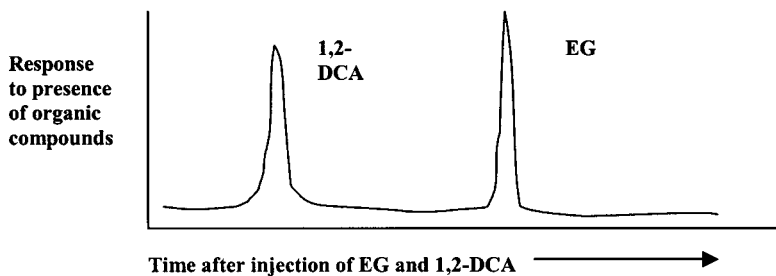
Compound	<i>T</i> (mp) (°C)	<i>T</i> (bp) (°C)	Soluble in
HOCH ₂ CH ₂ OH	−12.6	197.3	Polar solvents
ClCH ₂ CH ₂ Cl	−35.7	83.5	Nonpolar solvents

the headspace, whereas any attempt to extract EG from an aqueous solution that contains dissolved EG is useless! Because both compounds are liquids at room temperature, they do exhibit sufficient vapor pressure to be said to be amenable to analysis by gas chromatography. However, it may prove difficult to “chromatograph” them on the same column. The fundamental differences between a hydroxyl covalently bonded to carbon versus a chlorine atom bonded to carbon become evident when one attempts to separate the two. We will continue to use the physical–chemical differences between EG and 1,2-DCA to develop the concept of a separation between the two compounds by differential migration through a hypothetical “column” and through a series of consecutive stages known as the Craig distribution.

2. WHAT IS DIFFERENTIAL MIGRATION ANYWAY?

As first observed in 1903 by M. Tswett, a Russian botanist, when plant pigments were dissolved in a nonpolar solvent such as hexane and this solution was passed through a glass column packed with calcium carbonate, a separation of the two major forms of chlorophyll occurred due to a differential migration through the packed “stationary phase.” This observation of “color writing” led to the most used term in the separation sciences today—chromatography!

If a mixture containing EG and 1,2-DCA is introduced into a column, it is possible to conceive of the notion that the molecules that make up each compound would migrate differentially through the packed bed or stationary phase. Let us assume that this hypothetical column tends to retain the more polar EG longer. This separation of EG from 1,2-DCA is shown as follows:



We observe that the dispersion of the molecules as represented by σ^2 is found to be proportional to the distance migrated, z , according to:

$$\sigma^2 = kz$$

where k , the constant of proportionality, depends on the system parameters and operating conditions. Because k is a ratio of the degree of spread to migration distance, k can be referred to as a “plate height.” The resolution, R_s , between the separated “peaks” can be defined in terms of the distance between the apex of the peaks and the broadening of the peak according to

$$R_s = \frac{\Delta z}{\tau \sigma}$$

where τ is defined as being equal to 4 (10). We will have more to say on this topic later. It also becomes evident that Δz is proportional to the migration distance z and σ is proportional to the square root of the migration distance. Expressed mathematically, we have:

$$\Delta z \propto z$$

and

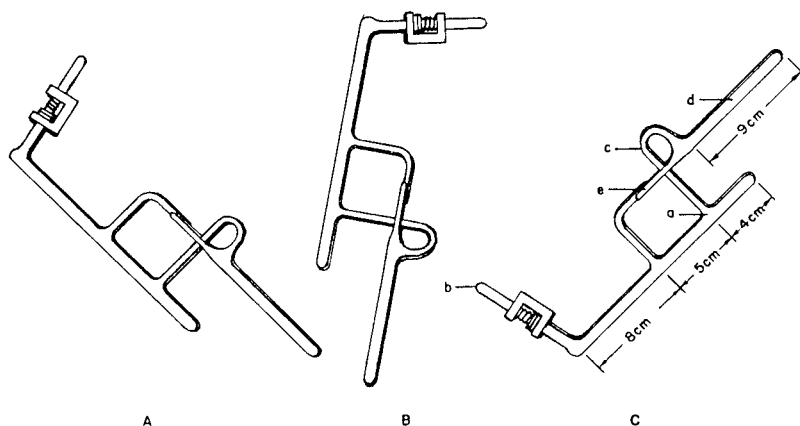
$$\sigma \propto \sqrt{z}$$

These equations tell us that the distance between zone centers increases more rapidly than the zone widths. From the definition of R_s , this suggests that resolution improves with migration distance. We will have more to say about resolution when we take up chromatography. Differences in the rates of analyte migration, however, do not explain the fundamental basis for separating EG from 1,2-DCA. For this, we begin by discussing the principles that underlie the Craig countercurrent extraction experiment.

3. WHAT CAUSES THE BANDS TO SEPARATE?

We just saw that, experimentally, EG and 1,2-DCA differentially migrate through a stationary phase when introduced into a suitable mobile phase and that chromatography arises when this mobile phase is allowed to pass through a chemically selective stationary phase. It is not sufficient to merely state that EG is retained longer than 1,2-DCA. It is more accurate to state that EG partitions to a greater extent into the stationary phase than does

1,2-DCA largely based on “like-dissolves-like.” The stationary phase is more like EG than 1,2-DCA due to similar polarity. This is all well and good, yet these statements do not provide enough rationale to establish a true physical–chemical basis for separation! In Chapter 3, we introduced liquid–liquid extraction (LLE) and also considered successive or multiple LLE. What we did not discuss is what arises when we transfer this immiscible upper or top phase or layer to a second sep funnel (first Craig stage or $n = 1$, see below). Prior to this transfer, the second sep funnel will already contain a fresh lower phase. Equilibration is allowed to occur in the second sep funnel while fresh upper phase is brought in contact with the lower phase in the first funnel. What happens if we then transfer the upper phase from this second sep funnel to a third sep funnel that already contains a fresh lower phase? This transfer of the upper phase in each sep funnel to the next “stage” with subsequent refill of the original sep funnel with a fresh upper phase can be continued so that a total of n stages and $n + 1$ sep funnels are used. It becomes very tedious to use sep funnels to conduct this so-called countercurrent extraction. A special glass apparatus developed by L. C. Craig in 1949 provides a means to perform this extraction much more conveniently. Twenty or more Craig tubes are connected in series in what is called a “Craig machine.” Once connected, up to 1000 tubes previously filled with a lower phase can participate in countercurrent extraction by a mere rotation of the tubes. The following is a schematic diagram of a single Craig tube of 2 mL capacity undergoing rotation:



It is this rotational motion that removes the extracted organic phase while a fresh organic phase is introduced back into the tube. The phases separate in position A, and after settling, the tube is brought to position B. Then, all of

the upper phase flows into decant tube d through c, as the lower phase is at a. When the tube is brought to position C, all of the upper phases in the decant tube are transferred through e into the next tube, and rocking is repeated for equilibration. The tubes are sealed together through the transfer tube, location e in the figure, to form a unit. These units are mounted in series and form a train having the desired number of stages (11).

The Craig countercurrent extraction enables one to envision the concept of discrete equilibria and helps one understand how differences in partition coefficients among solutes in a mixture can lead to separation of these solutes. Consider a cascade of $n + 1$ stages, each stage containing the same volume of the lower phase. We seek to explain the foreboding looking Table 4.2. Let us also assume that the total amount of solute is initially introduced into stage 0 (i.e., the first Craig tube in the cascade). The solute is partitioned between the upper and lower phases, as we saw in Eq. (3.16), represented here according to

$$p = \frac{VD}{1 + VD} \quad (4.1)$$

$$q = \frac{1}{1 + VD} \quad (4.2)$$

where p is the fraction of total solute that partitions into the upper phase and q is the fraction of total solute that partitions into the lower phase. Also, by definition, the following must be true:

$$p + q = 1$$

V is the ratio of the upper phase volume to the lower phase volume and is usually equal to 1 because both volumes in the Craig tubes are usually equal. D is the distribution ratio and equals K_D , the molecular partition coefficient in the absence of secondary equilibria, as discussed in Chapter 3. If we introduce a mixture of solutes to stage 0, each solute will have its own value for D and hence a unique value for p and q . For example, for a mixture containing four solutes, we would realize a fraction p for the first solute, a fraction p' for the second solute, and so forth. In a similar manner, we would also realize a fraction q for the first solute, a fraction q' for the second solute, and so forth.

We seek now to show how the ps and qs of Table 4.2 were obtained. We also wish to show how to apply the information contained in Table 4.2. We then extrapolate from the limited number of Craig tubes in Table 4.2 to

TABLE 4.2 Countercurrent Distribution of a Given Solute in a Craig Apparatus

	Stage				Distribution
	0	1	2	3	4
Introduce solute and equilibrate	p/q				
Total	1				1
First transfer	$0/q$	$p/0$			
Total	q	p			$(q + p)^1$
Equilibrate	$p(q)/q(p)$	$p(p)/q(p)$			
Second transfer	$0/q(q)$	$p(q)/q(p)$	$p(p)/0$		
Total	q^2	$2pq$	p^2		$(q + p)^2$
Equilibration	$p(qq)/q(qq)$	$p(2pq)/q(2pq)$	$p(pp)q(p^2)$		
Third transfer	$0/q^3$	$pq^2/2q^2p$	$2p^2q/qp^2$	$p^3/0$	
Total	q^3	$3pq^2$	$3p^2q$	p^3	$(q + p)^3$
Equilibrate	$p(q^3)/q(q^3)$	$p(3pq^2)/q(3pq^2)$	$p(3p^2q)/q(3p^2q)$	$p(p^3)/q(p^3)$	
Fourth transfer	$0/q^4$	$pq^3/3q^3p$	$3p^2q^2/3p^2q^2$	$3p^3q/p^3q$	$p^4/0$
Total	q^4	$4q^3p$	$6p^2q^2$	$4p^3q$	p^4
					$(q + p)^4$

a much larger number of tubes and see what effect this increase in the number of Craig tubes has on the degree of resolution, R_s .

We start with a realization that once a mixture of solutes, such as our pair, EG and 1,2-DCA, is introduced into the first Craig tube, an initial equilibration occurs and this is shown at stage 0. Again, if p and q represent the fraction of EG in each phase, then p' and q' represent the fraction of 1,2-DCA in each phase. The first transfer involves moving the upper phase that contains a fraction p of the total amount of solute to stage 1. A volume of upper phase equal to that in the lower phase is now added to stage 0 and a fraction p of the total amount of solute in stage 0, q , is partitioned into the upper layer while a fraction q of the total, q , is partitioned into the lower phase. A fraction p of the total p remains in the upper layer in stage 1 and a fraction q of the total p is partitioned into the lower phase. The remainder of Table 4.2 is built by partition of a fraction p of the total after each transfer and equilibration to the upper layer and by partition of a fraction q of the total into the lower layer. The last column in Table 4.2 demonstrates that if each row labeled "total" is added, this sum is the expansion of a binomial distribution, $(q + p)^r$, where r is the number of transfers. The fraction of solute in each n th stage after the r th transfer and corresponding equilibration can then be found using:

$$f = \frac{r!}{n!(r-n)!} p^n q^{r-n} \quad (4.3)$$

This fraction represents the sum of the fractions in the upper and lower phases for that stage. For example, suppose we wish to predict the fraction of EG and the fraction of 1,2-DCA in stage 3 after four transfers. Let us assume that the upper phase is the less polar phase. Let us also assume that the distribution ratio for 1,2-DCA into the less polar upper phase is favored and that $D_{1,2-DCA} = 4$. Let us also assume that EG prefers the more polar lower phase and has a distribution ratio that favors the lower phase and that $D_{EG} = 0.1$. We also assume that the volume of upper phase equals that of the lower phase (i.e., $V_{upper} = V_{lower}$) and the volume ratio is, therefore, 1. Upon substituting into Eq. (4.3) without considering values for p and q yields:

$$f^{3,4} = \frac{4!}{3!(4-3)!} p^3 q = 4p^3 q$$

A comparison of this result with that for the total fraction in the third stage after the fourth transfer (refer to Table 4.2) shows this result to be identical

to that shown in the table. Next, we proceed to evaluate p and q for each of the two solutes. Using Eqs. (4.1) and (4.2), we find the following:

$$p(1, 2\text{-DCA}) = 0.9, \quad p(\text{EG}) = 0.0909$$

$$q(1, 2\text{-DCA}) = 0.1, \quad q(\text{EG}) = 0.909$$

Upon substituting these values for p and q for each of the two solutes in the mixture into Eq. (4.3), we obtain:

$$f_{1,2\text{-DCA}}^{3,4} = (4)(0.5)^3(0.1) = 0.292$$

$$f_{\text{EG}}^{3,4} = (4)(0.0909)^3(0.909) = 0.00273$$

where $f_{1,2\text{-DCA}}^{3,4}$ is the fraction of 1,2-DCA present in the third stage after four transfers and $f_{\text{EG}}^{3,4}$ is the fraction of EG present in the third stage after four transfers. Hence, after four transfers, we find the fraction of 1,2-DCA in stage 3 (i.e., upper and lower phases) to be 0.292, whereas the fraction of EG in stage 3 is only 0.00273. The fact that these fractions are so different in magnitude is the basis for a separation of 1,2-DCA from EG.

4. WHAT HAPPENS IF WE REALLY INCREASE THE NUMBER OF CRAIG TUBES?

We have just examined a relatively small number of Craig countercurrent extractions and seen that differences in D or K_D among solutes results in different distributions among the many Craig tubes. Most distributions are normally distributed. In the absence of systematic error, random error in analytical measurement is normally distributed and this assumption formed the basis of much of the discussion in Chapter 2. A continuous random variable x has a Normal distribution with certain parameters μ (mean, parameter of location) and σ^2 (variance, parameter of spread) if its density function is given by (12):

$$f(x; \mu, \sigma) = \frac{1}{\sigma\sqrt{2\pi}} \exp\left(-\frac{1}{2} \frac{(x - \mu)^2}{\sigma^2}\right)$$

The binomial distribution in Eq. (4.3) closely approximates a Gaussian distribution when r and n are large. We can write the distribution as a continuous function of the stage number as

$$f \approx \frac{1}{\sqrt{rpq}\sqrt{2\pi}} \exp - \left(\frac{1}{2} \frac{(n - rp)^2}{rpq} \right) \quad (4.4)$$

Equation (4.4) is a very good approximation when the total number of stages is larger than 20 or when the product rpq is greater than or equal to 3. Comparing Eq. (4.4) to the above classical relationship for a Gaussian or Normal distribution leads to the following observation for the standard deviation, σ , of the distribution:

$$\sigma \approx \sqrt{rpq} \quad (4.5)$$

The mean Craig stage is also that stage with the maximum fraction. This distribution mean is given by

$$\mu = rp \quad (4.6)$$

Equation (4.6) enables a calculation of the mean in this Craig counter-current distribution and Eq. (4.5) yields an estimate of the standard deviation in the distribution among Craig tubes. For example, let us return to our two solutes, 1,2-DCA and EG. Earlier, we established values for p and q from a knowledge of D or, in the limit of purely molecular partitioning, K_D . Let us find μ and σ for both compounds after 100 transfers (i.e., $r = 100$) have been performed:

$$D(1, 2\text{-DCA}) = 4, \quad D(\text{EG}) = 0.1$$

$$p(1, 2\text{-DCA}) = 0.8, \quad p(\text{EG}) = 0.0909$$

$$q(1, 2\text{-DCA}) = 0.2, \quad q(\text{EG}) = 0.909$$

Upon substituting these values into Eq. (4.5) we obtain for 1,2-DCA,

$$\sigma_{1,2\text{-DCA}} = \sqrt{rpq} = \sqrt{(100)(0.8)(0.2)} = 4$$

Upon substituting these values into Eq. (4.6), we obtain for 1,2-DCA,

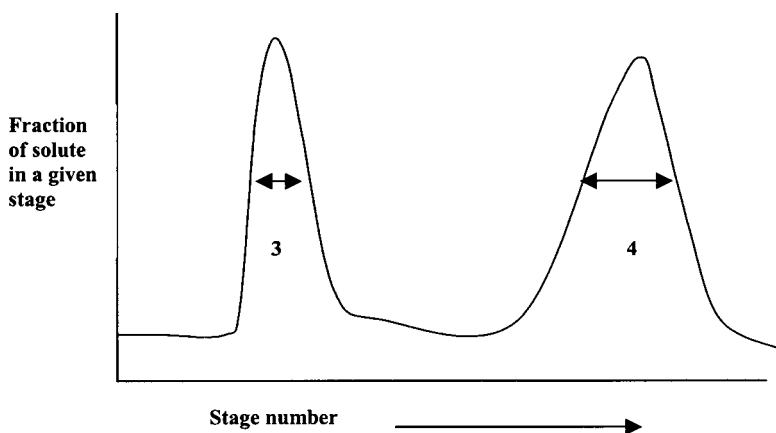
$$\mu_{1,2\text{-DCA}} = rp = (100)(0.8) = 80$$

In a similar manner, for EG we obtain

$$\sigma_{EG} = \sqrt{(100)(0.01)(0.91)} = 2.9$$

$$\mu_{EG} = (100)(0.091) = 9.1$$

It becomes obvious now that differences in D or K_D result in different stages in which the maximum fraction appears. The degree of band broadening is also larger for the solute with the larger value of μ . When the fraction of solute in a given stage is plotted against the stage number, Gaussian-like distributions are produced. The following is a sketch of such a plot:



It appears that 100 transfers using a Craig countercurrent apparatus enabled a more than adequate separation of EG and 1,2-DCA. We have, therefore, found a way to separate organic compounds! Before we leave the countercurrent separation concept, let us discuss the significance of Eqs. (4.5) and (4.6) a bit further. Equation (4.6) suggests that each solute migrates a distance equal to a constant fraction of the solvent front and Eq. (4.5) suggests that the width of the peak increases with the square root of the number of transfers. Separation is achieved as the number of transfers increase. The distance that each peak travels is proportional to r and the width of the peak is proportional to the square root of r . It is instructive to compare these findings from countercurrent extraction to those of analyte migration discussed earlier. Differential migration and countercurrent extraction techniques serve to help us to begin thinking about separations. These techniques “set the stage” for the most powerful of separation methods, namely chromatography.

5. WHAT IS CHROMATOGRAPHY?

Harris states that chromatography is a “logical extension of countercurrent distribution” (14). Chromatographic separation is indeed the countercurrent extraction taken to a very large number of stages across the chromatographic column. The following quotation is taken from an earlier text (15):

Chromatography encompasses a series of techniques having in common the separation of components in a mixture by a series of equilibrium operations that result in the entities being separated as a result of their partitioning (differential sorption) between two different phases; one stationary with a large surface and the other, a moving phase in contact with the first.

The “inventors” of partition chromatography, Martin and Synge, in 1941 defined chromatography this way (16):

The mobile phase need not be a liquid but may be a vapour . . . Very refined separations of volatile substances should therefore be possible in a column in which permanent gas is made to flow over gel impregnated with a non-volatile solvent in which the substances to be separated approximately obey Raoult’s law.

The following quotation is titled “The King’s Companions—A Chromatographical Allegory ” (17):

A great and powerful king once ruled in a distant land. One day, he decided he wanted to find the ten strongest men in his kingdom. They would be his sporting companions and would also protect him. In return, the king would give them splendid chambers in his palace and great riches.

But how would these men be found? For surely thousands from his vast lands would seek this promise of wealth and power. From amongst these thousands, how would he find the ten very strongest?

The king consulted his advisors. One suggested a great wrestling tournament, but that would be too time consuming and complicated. A weight-lifting contest was also rejected. Finally, an obscure advisor named *Chromos* described a plan that pleased the king.

“Your majesty,” said *Chromos*, “you have in your land a mighty river. Use it for a special contest. At intervals along the river, have your engineers erect poles. The ends of each pole should be anchored on opposite banks so that each pole stretches across the river. The pole must be just high enough above the surface of the river for a man being carried along by the current to reach up and grab hold of it. So strong is the current that he will not be able to

pull himself out, but will just be able to hold on until, his strength sapped, the pole will be torn from his grasp. He will be carried downstream until he reaches the next pole which he will also grasp hold of. Of course, the weakest man will be able to hold on to each pole for the shortest length of time, and will be carried downstream fastest. The strongest man will hold on the longest, and will be carried along most slowly by the river. You have only to throw the applicants into the river at one particular place and measure how long it takes each man to get to the finish line downstream (where he will be pulled out). As long as you have enough poles spaced out between the start and finish, the men will all be graded exactly according to their strength. The strongest will be those who take the longest time to reach the finish line.”

So simple and elegant did this method sound, that the king decided to try it. A proclamation promising great wealth and power to the ten strongest men was spread throughout the kingdom. Men came to the river from far and wide to participate in the contest Chromos had devised and the contest was indeed successful. Simply and quickly, the combination of *moving* river and *stationary* poles separated all the applicants from one another according to their strength.

So the king found his ten strongest subjects, and brought them to his palace to be his companions and protectors. He rewarded them all with great wealth. But the man who received the greatest reward was his advisor, *Chromos*.

Do you see the analogy?

In Chapter 3, we have alluded to chromatographic separation as *the* most important separation technique to TEQA. Indeed, much of the innovative sample prep techniques for trace organics described in Chapter 3 are designed to enable a sample of environmental interest to be nicely introduced into a chromatograph. A chromatograph is an analytical instrument that has been designed and manufactured to perform either gas or liquid column chromatography.

Chromatography is a separation phenomenon that occurs when a sample is introduced into a system in which a mobile phase is continuously being passed through a stationary phase. Chromatography has a broad scope, in that small molecules can be separated as well as quite large ones. Of interest to TEQA is the separation, detection, and quantification of relatively small molecules. In Chapter 3, we introduced SPE as an example of frontal chromatography. In this chapter, we will discuss elution chromatography exclusively because this form of chromatographic separation

lends itself to instrumentation. We will also limit our discussion of chromatography to column methods while being fully aware of the importance of planar chromatography, namely paper and thin-layer chromatography, because our interest is in trace chromatographic analysis. We will further limit our discussion to the two major types of chromatography most relevant to TEQA, namely gas chromatography (GC) and high-performance liquid chromatography (HPLC). Figure 4.1 represents an attempt to place the major kinds of chromatographic separation science in various regions of a two-dimensional plot whereby analyte volatility increases from low to high along the ordinate, whereas analyte polarity increases from left to right along the abscissa. The reader should keep in mind that there is much overlap of these various regions and that the focus of the plot is on the use of chromatography as a separation concept without reference to the kinds of detector required. The horizontal lines denote regions where GC is not appropriate. The arrow pointing downward within the GC region serves to point out that the demarcation between volatile analytes and semivolatile ones is not clear-cut. This plot reveals one of the reasons why GC has been so dominant in TEQA while revealing just how limited GC as a determinative technique really is! This plot also reveals the more universal nature of HPLC in comparison to GC.

6. WHY IS GC SO DOMINANT IN TEQA?

There are several reasons for this and GC is still the dominant analytical chromatographic determinative technique used in environmental testing labs today. Let us construct a list of reasons why:

- Gas chromatography was historically the first “instrumented” means to separate organic compounds and was first applied in the petroleum industry.
- Gas chromatography continuously evolved from the packed column, where resolution was more limited to capillary columns that significantly increase chromatographic resolution.
- The EPA organics protocol (refer to Fig. 1.2), classifies priority pollutant organics based on the degree of volatility and the volatile and lower-molecular-weight semivolatile regions of Fig. 4.1 are predominantly GC.
- Gas chromatography is simpler to comprehend in contrast to HPLC largely because the mobile phase in GC is chemically inert and contributes nothing to analyte retention and resolution.
- Detectors in GC can be of low, medium, and high sensitivity; highly sensitive GC detectors are of utmost importance to TEQA.

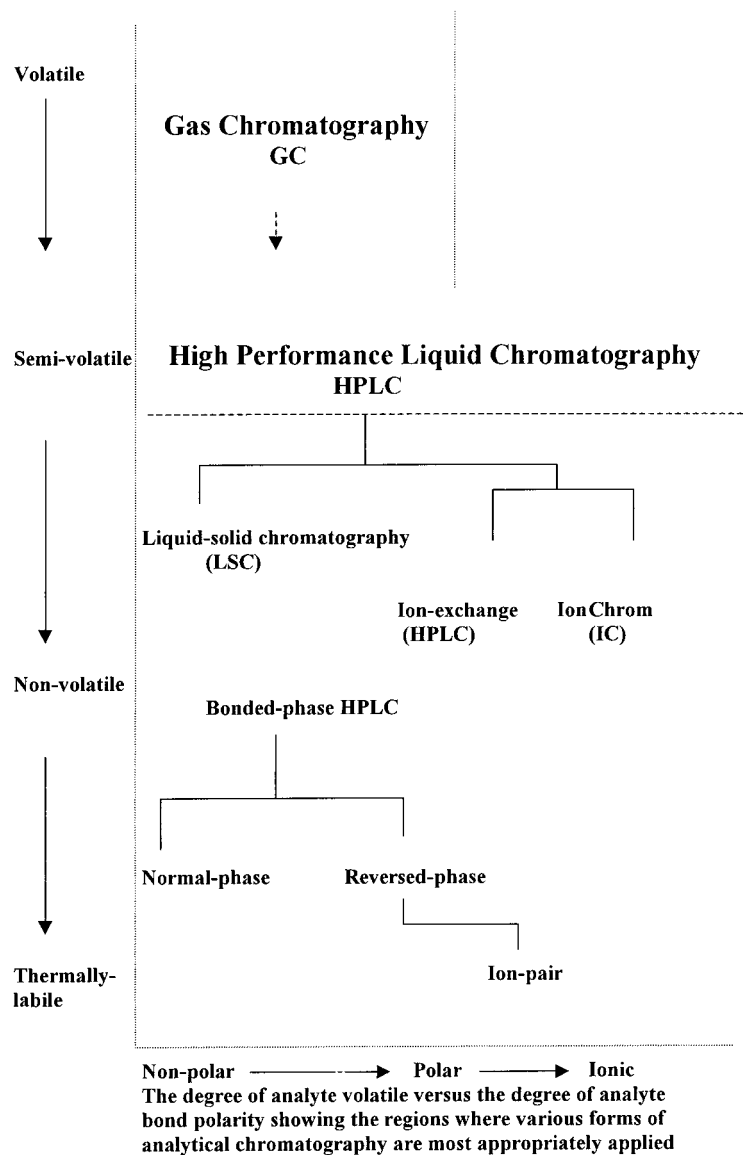


FIGURE 4.1 Degree of analyte volatility versus degree of analyte polarity.

- The price and size of a GC–MS instrument has declined over the past decade and the instrument has become more sensitive and more robust.

7. WHY IS HPLC MORE UNIVERSAL IN TEQA?

There are several reasons for this:

- High-performance liquid chromatography occupies a much larger region of Fig. 4.1 and this fact suggests that a much larger range of organic compounds are amenable to analysis by HPLC in contrast to GC.
- High-performance liquid chromatography can take on several different forms depending on the chemical nature of the mobile phase and the stationary phase, respectively; this leads to a significant rise in the scope of applications.
- Chemical manipulation of the mobile phase in HPLC enables gradient elution to be conducted.
- The analyte of interest remains dissolved in the liquid phase and can be thermally labile, unlike GC whereby an analyte must be vaporized and remain thermally stable.

8. CAN WE VISUALIZE A CHROMATOGRAPHIC SEPARATION?

Yes we can! Figure 4.2 is a hypothetical separation of a three-component mixture. Introduction of the mixture is diagrammatically shown in snapshot 1; elution of the mixture with a mobile phase begins in snapshot 2. Snapshots 3 and 4 depict the increase in chromatographic resolution, R_s , as the mixture moves through the column. Four chief parameters are used to characterize a chromatographic separation: the distribution coefficient, retention or capacity factor, selectivity and column efficiency, or the number of theoretical plates. We will spend the next few paragraphs developing the mathematics underlying chromatographic separation.

9. CAN WE DEVELOP USEFUL MATHEMATICAL RELATIONSHIPS FOR CHROMATOGRAPHY?

Yes we can! Let us begin by first recognizing that an analyte that is introduced into a chromatographic column, much like that shown in Fig. 4.2, distributes itself between a mobile phase, m , and a stationary phase, s , based

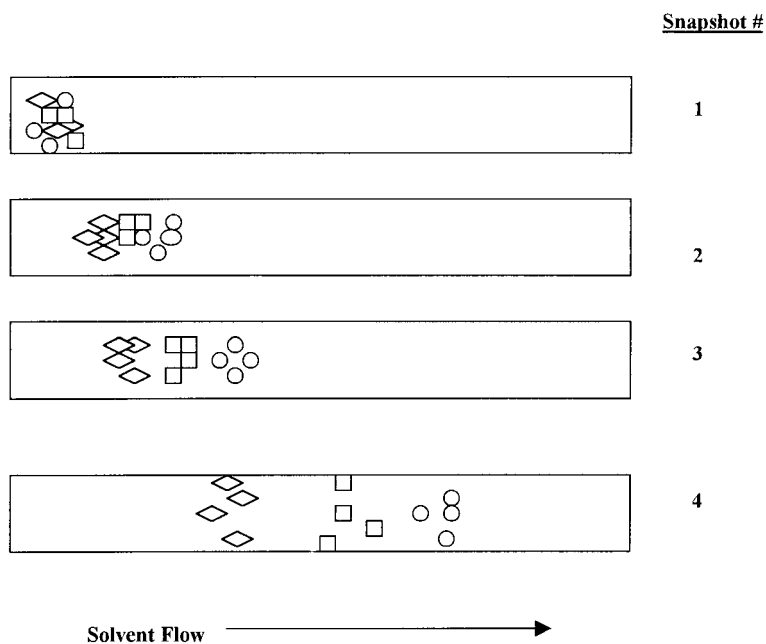


FIGURE 4.2 Hypothetical separation of a mixture of three chemically different substances via chromatography along with important terms.

on the amount of analyte distributed instead of the concentration distributed. The fraction of the i th analyte in the stationary phase, ϕ_i^s , is defined as

$$\phi_i^s = \frac{\text{amt}_i^s}{\text{amt}_i^m} = \frac{C_i^s V^s}{C_i^m V^m + C_i^s V^s} \quad (4.7)$$

where C_i^m and C_i^s are the i th analyte concentrations in both phases. V^m and V^s are the volumes of both phases. Let us define a molecular distribution constant for this i th analyte K_i , as

$$K_i = \frac{C_i^s}{C_i^m} \quad (4.8)$$

Equation (4.8) can be substituted into Eq. (4.7) to yield the fraction of the i th analyte in terms of the molecular distribution constant and ratio of phase volumes according to

$$\phi_i^s = \frac{K_i V^s / V^m}{1 + K_i V^s / V^m} \quad (4.9)$$

We now define the capacity factor, k' , a commonly used parameter in all of chromatography, as a ratio of the amount of analyte i in the stationary phase to the amount of analyte i in the mobile phase at any one moment:

$$k' = \frac{\text{amt}_i^s}{\text{amt}_i^m} = \frac{C_i^s V^s}{C_i^m V^m} = K_i \left(\frac{V^s}{V^m} \right) \quad (4.10)$$

As we discussed extensively in Chapter 3, when secondary equilibria are involved, K is replaced by D . Combining Eqs. (4.9) and (4.10) gives a relationship between the capacity factor and the fraction of analyte i distributed into the stationary phase:

$$\phi_i^s = \frac{k'}{1 + k'} \quad (4.11)$$

also, the fraction ϕ_i^m of analyte i in the mobile phase is

$$\phi_i^m = \frac{1}{1 + k'} \quad (4.12)$$

Equation (4.12) gives the fraction of analyte i , once injected into the flowing mobile phase, as it moves through the chromatographic column. This analyte migrates only when in the mobile phase. The velocity of the analyte through the column, v_s , is a fraction of the mobile phase velocity v according to

$$v_s = v\phi_i^m \quad (4.13)$$

Equation (4.13) suggests that the analyte does not migrate at all ($v_s = 0$) and when $\phi_i^m = 1$. It also suggests that the analyte moves with the same velocity as the mobile phase ($v_s = v$). The analyte velocity through the column equals the length, L , of the column divided by the analyte retention time, t_R :

$$v_s = L/t_R$$

The velocity of the mobile phase is given by the length of column divided by the retention time of an unretained component, t_0 , according to

$$v = L/t_0$$

Substituting for v_s and v into Eq. (4.13) yields

$$t_R = \frac{t_0}{\phi^m} \quad (4.14)$$

The mobile-phase volumetric flow rate, F , expressed in units of cubic centimeters per minute or milliliters per minute and is usually fixed and unchanging in chromatographic systems. Thus, because $t_R = V_R/F$ and $t_0 = V_0/F$, Eq. (4.14) can be rewritten in terms of retention volumes:

$$V_R = \frac{V_0}{\phi^m}$$

The retention volume, V_R , for a given analyte can be seen as the product of two terms V_0 and the reciprocal of ϕ^m :

$$= V_0 \left(\frac{1}{\phi^m} \right) \quad (4.15)$$

Substituting Eq. (4.12) into the above relationship gives

$$= V_0 \left(\frac{1}{1/(1+k')} \right)$$

Upon rearranging and simplifying,

$$V_R = V_0 [1 + k'] \quad (4.16)$$

Equation (4.16) has been called *the* fundamental equation for chromatography. Each and every analyte of interest that is introduced into a chromatographic column will have its own capacity factor, k' . The column itself will have a volume V_0 . The retention volume for a given analyte is then viewed in terms of the number of "column volumes" passed through the column before the analyte is said to "elute." A chromatogram then consists of a plot of detector response versus t_R , where each analyte has a unique retention time if sufficient chromatographic resolution is provided. Hence, with reference to a chromatogram, the capacity factor becomes

$$k' = \frac{t_R - t_0}{t_0} \quad (4.17)$$

Figure 4.3 is an illustrative chromatogram that defines what one should know when examining either a GC or and an HPLC chromatogram. Because the two peaks shown in Fig. 4.3 have different retention times, their capacity factors differ according to Eq. (4.17). The ratio of two capacity factors, α , relates to the degree that a given chromatographic column is selective:

$$\alpha = \frac{k'_2}{k'_1} \quad (4.18)$$

Let us assume that we found a column that retains the more polar EG with respect to 1,2-DCA. Equation (4.13) would suggest that the $\phi_{1,2\text{-DCA}}^m$ is much larger than ϕ_{EG}^m . The unretained solute peak in the chromatogram, often called the chromatographic dead time or dead volume, might be due to the presence of air in GC or a solvent in HPLC. The adjusted retention volume, V'_R , and adjusted retention time, t'_R , are defined mathematically as

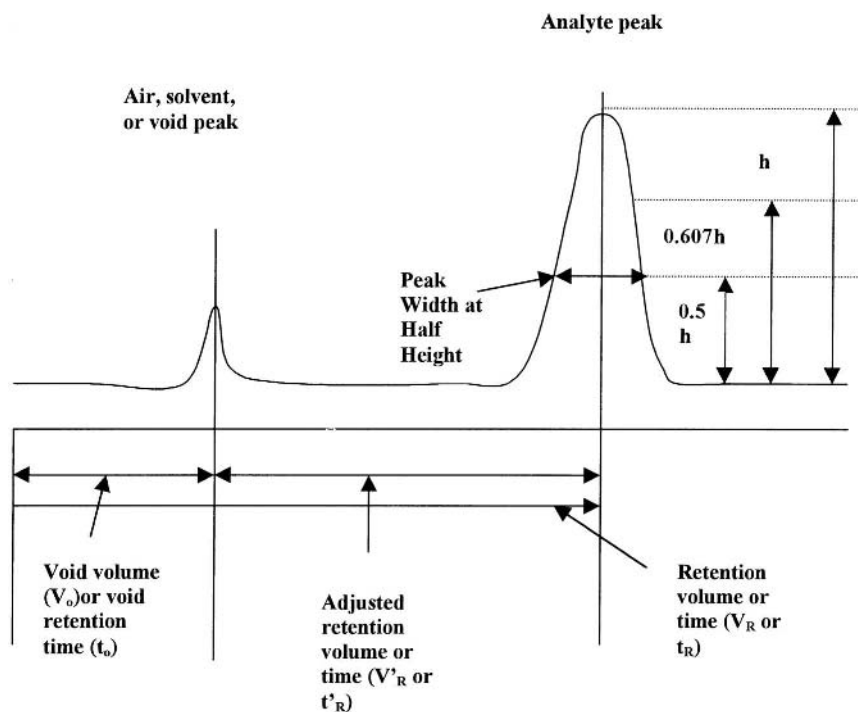


FIGURE 4.3 A typical GC or HPLC chromatogram with definitions.

$$V_R' = V_R - V_0$$

$$t_R' = t_R - t_0$$

We need now to go on and address the issue of peak width. As was pointed out earlier, the longer an analyte is retained in a chromatographic column, the larger is the peak width. This is almost a universal statement with respect to chromatographic separations.

10. HOW DOES ONE CONTROL THE CHROMATOGRAPHIC PEAK WIDTH?

The correct answer is to minimize those contributions to peak broadening! These factors are interpreted in terms of contributions to the height equivalent to a theoretical plate (HETP). This line of reasoning leads to the need to define a HETP that, in turn, requires that we introduce the concept of a theoretical plate in chromatography. So let us get started!

The concept of a theoretical plate is rooted in both the theory of distillation and in the Craig countercurrent extraction. A single distillation plate is a location whereby a single equilibration can occur. We already discussed the single equilibration that occurs in a single Craig stage. Imagine an infinite number of stages and we begin to realize the immense power of chromatography as a means to separate chemical substances. An equilibration of a given analyte between the mobile phase and stationary phase requires a length of column and this length can be defined as H . A column would then have a length L and a number of these equilibrations denoted by N , the number of theoretical plates. Hence, we define the HETP, abbreviated H for brevity here, as follows:

$$H = \frac{L}{N} \quad (4.19)$$

The number of theoretical plates in a given column, N , is mathematically defined as the ratio of the square of the retention time, t_R , or the retention volume, V_R (note that this is the apex of the Gaussian peak), of a particulate analyte of interest over the variance of that Gaussian peak. Expressed mathematically,

$$N = \left(\frac{t_R}{\sigma_t} \right)^2 = \left(\frac{V_R}{\sigma_V} \right)^2 \quad (4.20)$$

The number of theoretical plates can be expressed in terms of the width of the Gaussian peak at the base. This is expressed in units of time, t_w , where it is assumed that t_w approximates four standard deviations or, mathematically, $t_w = 4\sigma_t$, so that upon substituting for σ_t ,

$$N = \frac{t_R^2}{(t_w/4)^2} = 16 \left(\frac{t_R}{t_w} \right)^2 \quad (4.21)$$

Columns that significantly retain an analyte of interest (i.e., have a relatively large t_R) and also have a narrow peak width at base, t_w , must have a large value for N according to Eq. (4.21). Columns with large values for N such as from 1000 to 10,000 theoretical plates are therefore considered to be highly efficient. Many manufacturers prefer to cite the number of theoretical plates per meter instead of just the number of theoretical plates. The concept that the number of theoretical plates for a column (be it a GC or an HPLC column) can be calculated from the experimental GC or HPLC chromatogram is an important practical concept! In the realm of GC, when open tubular columns or capillary replaced packed columns, it was largely because of the significant difference in N offered by the former type of column. The second most useful measurement of N is to calculate N from the width of the peak at half-height, $t_w^{1/2}$, using the equation

$$N = 5.55 \left(\frac{t_R}{t_w^{1/2}} \right)^2 \quad (4.22)$$

It is up to the user as to whether Eq. (4.21) or (4.22) is used to estimate N . Figure 4.3 defines the peak width at half-height and the peak width at the base. When a GC or HPLC column is purchased from a supplier, pay close attention to how the supplier calculates N . It is also recommended that a peak be chosen in a chromatogram to calculate N whose k' is around 5. To continue our discussion of peak broadening, we need to return to the height equivalent to a theoretical plate, H .

11. IS THERE A MORE PRACTICAL WAY TO DEFINE H ?

Equation (4.19) defines H in terms of column length and a dimensionless parameter N . We will now derive an expression for H in terms of the chromatogram in units of time. We start by considering a chromatographic column of length L to which a sample has been introduced. This sample will experience band broadening as it makes its way through the column. We know that the degree of band broadening denoted by σ has units of distance.

H can be defined as the ratio of the variance, in units of distance, over the column length according to

$$H = \frac{\sigma^2}{L} \quad (4.23)$$

This dispersion in distance units can be converted to time units by recognizing that

$$\sigma = \phi^m v \sigma_t$$

Upon substituting for σ and substituting into Eq. (4.23) and doing some algebraic manipulation while recognizing that

$$t_R = \frac{L}{\phi^m v}$$

we now have an equation that relates H to the variance of the chromatographically resolved peak in time units according to

$$H = \frac{L \sigma_t^2}{t_R^2} \quad (4.24)$$

Equation (4.24) suggests that the height equivalent to a theoretical plate can be found from a knowledge of the length of the column, the degree of peak broadening as measured by the peak variance, in time units, and the retention time. We now discuss those factors that contribute to H because Eqs. (4.23) and (4.24) show that H is equal to the product of a constant and a variance. If we can identify those distinct variances, σ_i , that contribute to the overall variance, σ_{overall} , then these individual variances can merely be added. Expressed mathematically, for the i th independent contribution to chromatographic peak broadening, the statistics of propagation of error suggests that

$$\sigma_{\text{overall}}^2 = \sum_i \sigma_i^2$$

The concept that a rate theory is responsible for contributions to chromatographic peak broadening were first provided by van Deemter, Klinkenberg, and Zuiderweg. The random walk is the simplest molecular model and is due to Giddings (18).

12. WHAT FACTORS CONTRIBUTE TO CHROMATOGRAPHIC PEAK BROADENING?

It is important for the practicing chromatographer to understand the primary reasons why the mere injection of a sample into a chromatographic column will lead to a widening of the peak width. We alluded to peak broadening earlier when we introduced band migration. We will not provide a comprehensive elaboration of peak broadening. Instead, we introduce the primary factors responsible for chromatographic peak broadening. Following this, we introduce and discuss the van Deemter equation. The concept is termed chromatographic rate theory and is adequately elaborated on in the analytical literature elsewhere (19–21).

Equation (4.23) is the starting point for discussing those factors that broaden a chromatographic peak. By the time the solute molecules of a sample that have been injected into a column and have traveled a distance L , where L is the length of the GC or HPLC column, a Gaussian profile emerges. At the end of the column where the GC or HPLC detector is located, the peak has been broadened whereby one standard deviation has a length defined by $L - \sigma$ to the left of the peak apex at L and $L + \sigma$ to the right of the peak apex at L . H can now be thought of as the length of column, at the end of the column, that contains a fraction of analyte that lies between $L - \sigma$ and L (22). The fact that there exists a minimum H in a plot of H versus linear flow rate, u , suggests a complex mathematical relationship exists between H and u . The following factors have emerged:

- Multiple paths of solute molecules, the A term, present only in packed GC and HPLC columns and absent in open tubular GC columns. This term is also called eddy diffusion.
- Longitudinal diffusion, the B term, present in all chromatographic columns.
- Finite speed of equilibration, inability of solute molecules to truly equilibrate in one theoretical plate, the C term, present in all chromatographic columns. This term is also called the resistance to mass transfer term and, in more contemporary versions, consists of two mass transfer coefficients C_S , where S refers to the stationary phase, and C_M , where M refers to the mobile phase. Equilibrium is established between M and S so slowly that a chromatographic column always operates under nonequilibrium conditions. Thus, analyte molecules at the front of a band are swept ahead before they have time to equilibrate with S and thus be retained. Similarly, equilibrium is not reached at the trailing edge of a band, and molecules are left behind in S by the fast-moving mobile phase (23).

The above three factors broaden chromatographically resolved peaks by contributing a variance for each factor starting with Eq. (4.23) as follows:

$$H = \frac{1}{L}(\sigma^2) = \frac{1}{L} \left(\sum_i \sigma_i^2 \right)$$

$$H = H_L + H_S + H_M$$

where H_L is the contribution to H due to longitudinal diffusion, H_S is the contribution to H due to resistance to mass transfer to S , and H_M is the contribution to H due to resistance to mass transfer to M . We will derive only the case for longitudinal diffusion and state the other two without derivation.

13. HOW DOES LONGITUDINAL DIFFUSION CONTRIBUTE TO H ?

Molecular diffusion of an analyte of environmental interest in the direction of flow is significant only in the mobile phase. Its contribution, σ_L^2 , to the total peak variance can be found by substituting the molecular diffusivity and time into the Einstein equation:

$$\sigma^2 = 2Dt$$

On average, solute molecules spend the time $t = L/u$ in the mobile phase, so that the variance in the mobile phase is given as

$$\sigma_L^2 = \frac{2D_M L}{u}$$

where D_M is the solute diffusion coefficient in the mobile phase. The plate height contribution of longitudinal diffusion, H_L , is then obtained as

$$\begin{aligned} H_L &= \frac{\sigma^2}{L} = \frac{2\gamma D_M}{u} \\ &= \frac{B}{u} \end{aligned}$$

where γ is an obstruction factor that recognizes that longitudinal diffusion is hindered by the packing or bed structure. H_L is usually only a small contributor to H . However, when D_M is large and the mobile-phase velocity, u ,

is small, does H_L become significant? H_L is far more important in GC than in HPLC.

14. HOW DOES ALL OF THIS FIT TOGETHER?

Mass transfer into the stationary phase and the mobile-phase contribution to plate height contribute a term, C_S , and a term, C_M , respectively, to the total plate height in direct proportion to u . This is so because, unlike longitudinal diffusion, molecules diffuse at an angle of 90° with respect to the direction of the mobile-phase flow. The larger the mobile-phase velocity, the greater is the diffusion in this direction. Hence, we have a relationship between H and the linear mobile-phase velocity u according to

$$H = A + \frac{B}{u} + C_S u + C_M u \quad (4.25)$$

This is a more contemporary van Deemter equation and this equation takes on different contributions to the terms A , B , C_S , and C_M , depending on which form of chromatography is employed. We will introduce specific parameters that comprise both C terms when we discuss GC and HPLC. Equation (4.25) is plotted in Fig. 4.4. This plot is obtained by measuring H as the linear velocity of the mobile phase for the solute ethyl acetate dissolved in n -hexane and chromatographed on a normal-phase, silica-based HPLC column. The plot includes the eddy diffusion or the A term and shows the independence of eddy diffusion with respect to the mobile-phase flow rate. The plot shows the inverse relationship between H and u with respect to longitudinal diffusion. The plot shows the near linear relationship between H and u with respect to mass transfer in both phases. If we differentiate Eq. (4.25) with respect to u and set this derivative equal to zero, upon solving for u we find

$$u_{\text{optimum}} = \sqrt{\frac{B}{C}}$$

In practice, the mobile-phase linear velocity is set slightly higher than u_{optimum} so as to quicken the chromatographic run time (i.e., the time between injection and separation, and then detection). Figure 4.5 is a plot of H versus u for a packed GC column and for an open tubular GC column. H is much lower for the open tubular column because multiple flow paths are eliminated. Note also that the curvature in the plot in Fig. 4.5 for the open tubular column is much less than that of the packed column. This

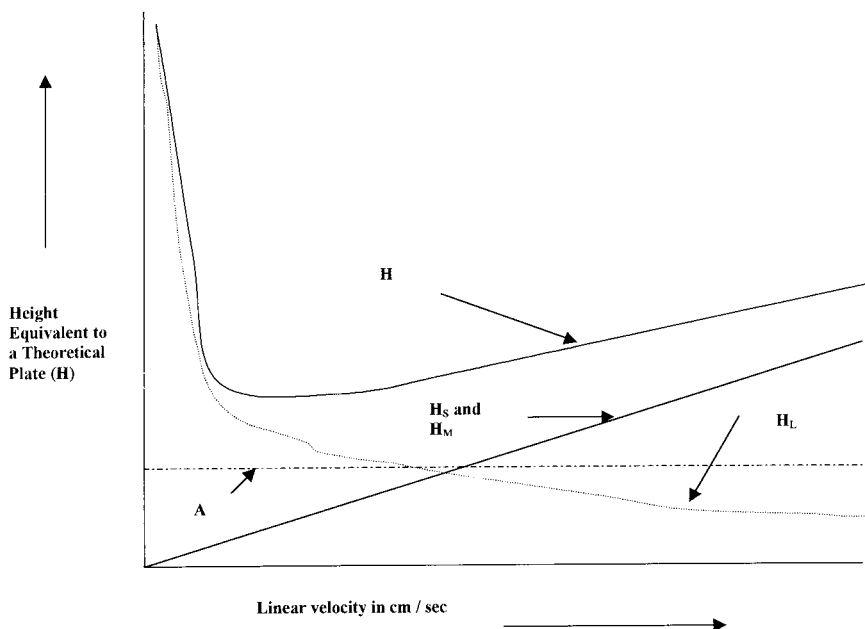


FIGURE 4.4 Plate height versus linear carrier gas velocity showing the distinct contributions to plate height.

much wider range of optimal mobile-phase velocities enables the chromatographer to use a much larger range of volumetric flow rates without sacrificing H . While we are discussing flow rates, we need to point out the significant difference between linear flow and volumetric flow with respect to column chromatography.

15. HOW DO WE DISTINGUISH BETWEEN LINEAR AND VOLUMETRIC FLOW RATES?

Note that Eq. (4.25) examines column efficiency as a complex function of linear mobile-phase velocity. It is the linear velocity that conducts analytes of interest through a chromatographic column to the detector. Any comparison of van Deemter curves such as that shown in Fig. 4.5 must use linear velocity because the influence of the column radius is eliminated. Jennings has articulated an interesting relationship between linear and volumetric flow rates, F , and incorporating the column radius, r_C , according to (24)

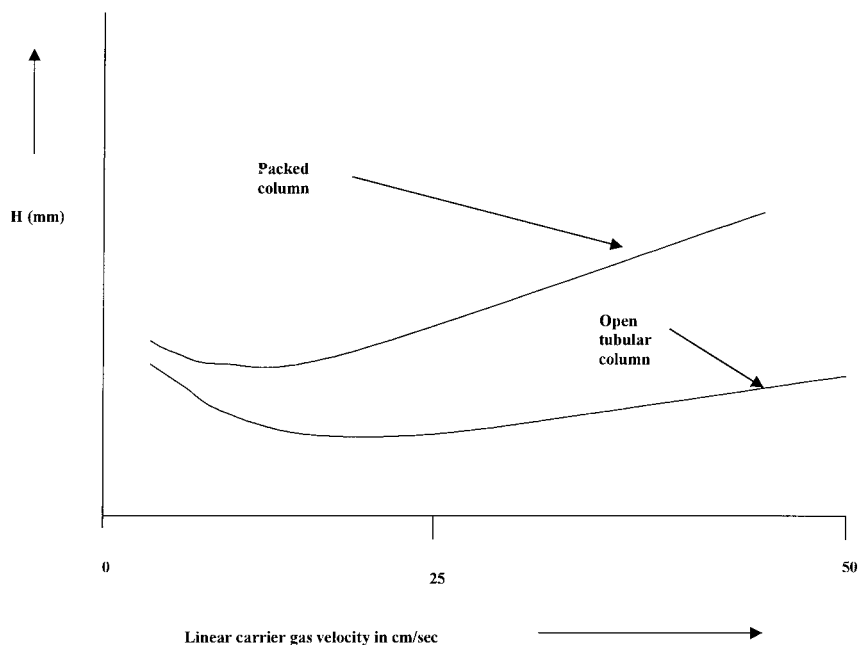


FIGURE 4.5 Plate height versus linear carrier gas velocity for a packed versus a capillary GC column.

$$u(\text{cm/s}) = \frac{1.67F(\text{cm}^3/\text{min})}{\pi r_C(\text{mm})^2} \quad (4.26)$$

Figure 4.5 reveals that with respect to the average linear velocity, the optimum linear velocity for a packed GC column is lower than that for an open tubular or capillary GC column. Equation (4.26) is used to calculate u for the five most commonly used GC column diameters and these results are shown in Table 4.3. The columns are listed from the smallest commercially available diameter to the largest, along with a representative volumetric flow rate passing through the column. It then becomes evident that open tubular columns exhibit linear flow rates that are three to five times higher than packed columns despite the fact that the user would have to replace compressed gas tanks less frequently! Linear flow rates can also be determined independently of Eq. (4.26) by measuring the retention time of an unretained component of the injected sample. In the case of GC, injection of methane or, as this author has done, injection of the butane vapor from a common cigarette lighter using a 10- μL liquid handling glass syringe gives

TABLE 4.3 Characteristics of Capillary GC Columns Having Different Internal Diameters

GC column	r_c (mm)	F (mL/min)	u (cm/s)
Capillary, 0.25 mm i.d.	0.125	0.75	35.6
Capillary, 0.32 mm i.d.	0.16	1.5	30.7
Capillary, 0.53 mm i.d.	0.265	5.0	38.0
Packed, $\frac{1}{8}$ in. o.d.	3.175	25	1.3
Packed, $\frac{1}{4}$ in. o.d.	6.35	75	1.0

t_0 . Knowing the length of the column, L , enables the linear velocity to be calculated according to

$$u = L/t_0$$

In reversed-phase HPLC with ultraviolet absorption detection (RP-HPLC-UV), this author has used as a source of an unretained component, the strongly absorbing and water-miscible solvent acetone. This measured retention time for acetone, commonly termed the “void or dead time,” can be used to calculate the linear velocity. Our understanding of what causes chromatographic peaks to broaden as they elute has led to the achievement of columns that maximize chromatographic resolution.

16. WHAT IS CHROMATOGRAPHIC RESOLUTION?

With reference to either a GC or HPLC chromatogram, the resolution, R_S , is defined as the ratio of the distance between retention times of two separated peaks to the average base peak width, t_w , of both peaks, 1 and 2. Expressed mathematically,

$$\begin{aligned}
 R_S &= \frac{t_R^2 - t_R^1}{\frac{1}{2}(t_w^1 + t_w^2)} \\
 &= \frac{2(t_R^2 - t_R^1)}{t_w^1 + t_w^2}
 \end{aligned}
 \tag{4.27}$$

Four chromatograms are presented in Fig. 4.6. It becomes visually obvious that resolution increases as one goes from top to bottom in the figure. In a manner similar to that used to derive Eq. (4.21), we recognize that the peak width at the base is equal to four times the standard deviation of the

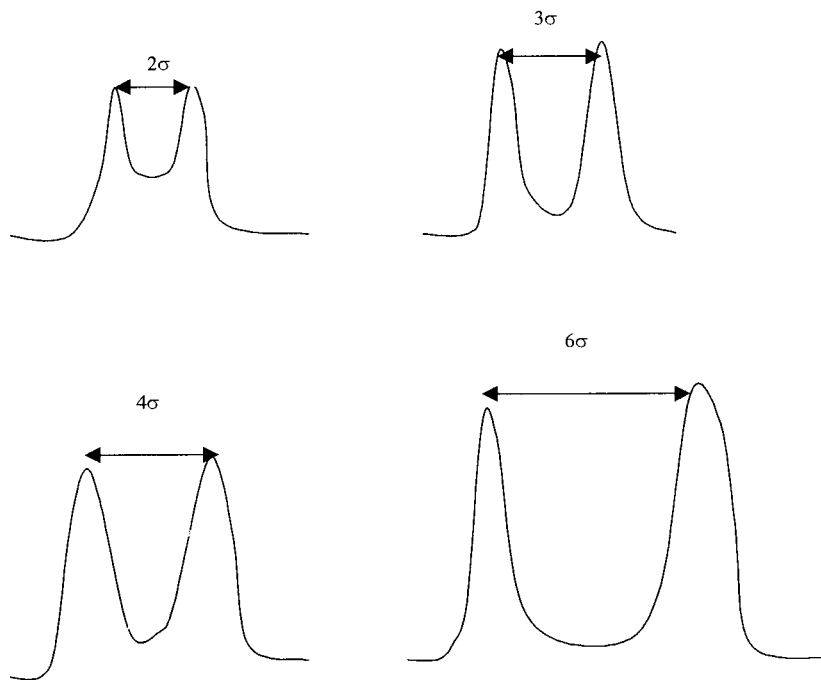


FIGURE 4.6 Chromatographic resolution and multiples of the peak standard deviation.

Gaussian peak profile (i.e., $t_w = 4\sigma_t$). We also assume that the variance of both peaks is equal so that $\sigma_\tau^1 = \sigma_\tau^2 = \sigma_\tau$. Upon substituting this into Eq. (4.27), we get

$$R_S = \frac{2(t_R^2 - t_R^1)}{8\sigma_\tau} = \frac{t_R^2 - t_R^1}{4\sigma_\tau} \quad (4.28)$$

Equation 4.28 shows that the larger the disengagement of a pair of chromatographically separated peaks, the greater the resolution. Equation (4.28) also shows that the smaller the variance of a peak, the greater the resolution. This equation can be viewed as a ratio of a change in retention time to the standard deviation, and different scenarios can be introduced. If we let Δt_R represent a change in the retention time for a given pair of peaks that have been partly or entirely resolved chromatographically, we can rewrite Eq. (4.28) to give

$$R_S = \frac{\Delta t_R}{4\sigma_t} \quad (4.29)$$

Equation (4.29) suggests that when $R_S = 0.5$, $\Delta t_R = 2\sigma_t$, it means that the distance in time units between both peak apexes equals two standard deviations of the Gaussian peak profile: When $R_S = 0.75$, $\Delta t_R = 3\sigma_t$; when $R_S = 1.0$, $\Delta t_R = 4\sigma_t$; and when $R_S = 1.5$, $\Delta t_R = 6\sigma_t$. Again, refer to Fig. 4.6, where each of these four different values for chromatographic resolution are shown. Hence, if we were to calculate a resolution whereby $R_S = 1.0$, Fig. 4.6 suggests that baseline resolution has not been attained. Computerized software has enabled a partially resolved chromatographic peak to be accurately quantitated and obviates the need for baseline resolution in many cases. Equations (4.28) and (4.29) are important definitions of R_S for a given situation. These relationships do not, however, show how R_S relates to the fundamental parameters of chromatographic separation. To establish this, we need to derive *the* fundamental resolution equation in column chromatography.

17. HOW DO YOU DERIVE THIS FUNDAMENTAL EQUATION?

Few elementary treatments of the topic of chromatography take the reader from all that we have developed so far to a consideration of exactly how resolution depends on chromatographic efficiency, selectivity, and capacity factor. We begin to do this here by following a derivation originally presented to the analytical literature by Karger et al. in their classic text on separation science (25). Let us start with Eq. (4.29), but before we do this, we need to find a way to incorporate N into this equation. Let us consider a separation of peaks 1 and 2 whose peak apexes are separated by Δt_R . Referring back to Eq. (4.20), we can rewrite this equation with reference to peak 2 for a retention time for peak 2 as t_2 and standard deviation, σ_2 , and solve for σ_2 to give

$$\sigma_2 = \frac{t_2}{\sqrt{N}}$$

Upon substituting for σ_2 back into Eq. (4.29), we obtain

$$R_S = \frac{\Delta t_R}{4(t_2/\sqrt{N})} = \frac{\sqrt{N}}{4} \left(1 - \frac{t_1}{t_2} \right)$$

At this point, we have the resolution in terms of the number of theoretical plates and retention times. We can proceed even further. By combining Eqs. (4.13) and (4.15) by eliminating ϕ^m , we obtain a relationship for retention times in terms of the capacity factor, k' , according to

$$\frac{t_R}{t_0} = 1 + k'$$

For peak 1, we have $t_1/t_0 = 1 + k'_1$ and likewise for peak 2, $t_2/t_0 = 1 + k'_2$ so that

$$\frac{t_1}{t_2} = \frac{1 + k'_1}{1 + k'_2}$$

We can now express resolution in terms of N and a ratio of capacity factors according to

$$\begin{aligned} R_S &= \frac{\sqrt{N}}{4} \left(1 - \frac{1 + k'_1}{1 + k'_2} \right) \\ &= \frac{\sqrt{N}}{4} \left(\frac{k'_2 - k'_1}{1 + k'_2} \right) \end{aligned}$$

Because $\alpha = k'_2/k'_1$, we can solve for $k'_1 = k'_2/\alpha$ and substitute for k'_1 above to get

$$R_S = \frac{\sqrt{N}}{4} \left(\frac{k'_2 - k'_2/\alpha}{1 + k'_2} \right)$$

Upon simplifying, we obtain

$$= \frac{\sqrt{N}}{4} \left(\frac{k'_2(1 - 1/\alpha)}{1 + k'_2} \right)$$

Upon rearranging terms, we arrive at *the* fundamental resolution equation:

$$R_S = \frac{\sqrt{N}}{4} \left(\frac{k'_2}{1 + k'_2} \right) \left(\frac{\alpha - 1}{\alpha} \right) \quad (4.30)$$

Equation (4.30) suggests that the degree of chromatographic resolution depends chiefly on three factors: N , k' , and α . N , the number of theoretical plates in a column, relates how efficient a chromatographic column is. N is

independent of the chemical nature of the analyte of interest. k'_2 , the capacity factor of the more retained component in a given pair, depends on the chemical nature of the analyte. k' , in general, is related to the product of the analyte's partition constant K and on the phase ratio β . The phase ratio in column chromatography is defined as the ratio of the stationary phase volume, V_s , to that of the mobile-phase volume, V_m . The stationary-phase volume takes on different values depending on how the stationary phase is defined with respect to the column support. Jennings has stated that with respect to GC, β is between 5 and 35 for packed GC columns and β is between 50 and 1000 for open tubular GC (26). In any event, the capacity factor for a given analyte can be calculated by knowing the partition constant and phase ratio according to

$$k' = K\beta = K \frac{V_s}{V_m} \quad (4.31)$$

α , the chromatographic separation factor between two adjacent peaks and often called, for simplicity, the column selectivity, relates to the ratio of k' values for both peaks of interest as introduced earlier. N can be changed by increasing or decreasing the column length, adjusting the flow rate to minimize H while maximizing N for a column of fixed length. Changes in α and k' are achieved by selecting different mobile- and stationary-phase chemical compositions or by varying the column temperature or, in some cases, the column pressure. k' can also be changed for a given component by changing the relative amounts of stationary versus mobile phase according to Eq. (4.31). Equation (4.30) shows that these three factors enter into the resolution equation in a complex manner. We now examine the mathematical nature of each term in Eq. (4.30).

18. WHAT IS EQ. (4.30) REALLY TELLING US?

The first implication of Eq. (4.30) is to realize that R_S approaches zero (i.e., no resolution between chromatographically resolved pairs of peaks) when N or k'_2 approaches zero or when α approaches 1. Note the nature of the third term in Eq. (4.30). As the magnitude of α increases, the contribution of the third term serves to increase R_S . The effect of the α term is shown in Table 4.4. The most significant gains in chromatographic resolution occur between values of α that are greater than 1, yet diminishing returns set in if α is raised above 5. A similar argument can be made for the effect of the second term in Eq. (4.30) (i.e., the k' term). The effect of the k' term is shown in Table 4.5. It appears that very small values of k' contribute little to increased R_S , whereas, again, diminishing returns are evident as k' is increased. The

TABLE 4.4 Chromatographic Selectivity and the Contribution of α to the Fundamental Resolution Equation

α	$\alpha - 1/\alpha$
1	0
1.1	0.091
1.5	0.333
2	0.5
5	0.8
10	0.9
20	0.95
100	0.99

greatest gains are found for a range of k' values between 2 and 5. Equation (4.30) also suggests that resolution varies with the square root of the number of theoretical plates. This is an important feature of Eq. (4.30) to remember! A mere doubling of the length of the column will only increase R_S by 1.4!

To summarize the discussion that led to Eq. (4.30), Karger et al. have stated it best (25):

Although each of these three parameters— N , α , and k'_2 —is important in controlling resolution, we usually do not attempt their simultaneous optimization in an actual separation. Experimental conditions are selected initially that favor large N values, within the practical limits of convenience plus reasonable separation times. Higher N values always provide improved resolution, other

TABLE 4.5 Chromatographic Capacity Factor and the Contribution of k' to the Fundamental Resolution Equation

k'	$k'/(1 + k')$
0.1	0.0909
0.5	0.333
1.0	0.500
2.0	0.666
5.0	0.833
10	0.909
20	0.952
50	0.980

factors being equal, and this is true of analytical or preparative separations, and simple or complex mixtures. With a reasonable starting value of N , adequate resolution will be attained in most cases if we optimize k'_2 approximately. In gas chromatography an optimum value of k'_2 can be achieved by varying the temperature. In liquid chromatography it is more profitable to vary systematically the composition of the mobile phase.

One other relationship is worth mentioning before we discuss the determinative techniques GC and HPLC. Equation (4.30) can be solved algebraically for the number of theoretical plates and this gives

$$N_{\text{required}} = 16R_S^2 \left(\frac{\alpha}{\alpha - 1} \right)^2 \left(\frac{k' + 1}{k'} \right)^2 \quad (4.32)$$

Equation (4.32) is of practical importance in that for a given resolution, the required number of theoretical plates can be found. To illustrate, let us calculate N_{required} for the chromatographic separation of two solutes with a given resolution $R_S = 1.5$ for two different values for the selectivity, $\alpha = 1.05$ and $\alpha = 1.10$ (27). The results of the application of Eq. (4.32) are shown in Table 4.6. For two solutes that have a value for $k' = 0.01$, the magnitude of the k' term in Eq. (4.32) is such as to require over a 100 million plates to realize a value for $\alpha = 1.05$. For two solutes that have a value for $k' = 1.0$, the magnitude of the k' term in Eq. (4.32) is such as to require over 50,000 plates to realize a value for $\alpha = 1.05$. For two solutes that have a

TABLE 4.6 Influence of k' on the Required Number of Theoretical Plates at Two Different Values for α for a Given R_S Applying Eq. (4.32)

k'	$[(k' + 1)/k']^2$	$N_{\text{required}} (\alpha = 1.05)$	$N_{\text{required}} (\alpha = 1.0)$
0.01	10,201	162,000,000	44,000,000
0.05	441	7,000,000	1,900,000
0.10	121	1,900,000	527,000
0.15	58.8	930,000	256,000
0.5	9	143,000	39,000
1.0	4	63,500	17,500
3.0	1.8	28,600	7,800
5.0	1.4	22,200	6,100
10	1.1	17,500	4,800
50	1.05	16,700	4,600

value for $k' = 10$, the magnitude of the k' term in Eq. (4.32) is such as to require over 10,000 plates to realize a value for $\alpha = 1.05$.

The fundamental theory of chromatographic separation has been presented in the broadest of terms. We next proceed to discuss *the* most common determinative technique for measuring trace concentration levels of organics in the environment, gas-liquid, or gas-solid chromatography.

19. HOW DOES A GC WORK?

A gas chromatograph provides the proper conditions for the separation and detection of trace organic compounds that have been isolated from the environment. A GC works by properly installing the instrument in a laboratory and understanding how to optimize the separation and detection of analytes of interest. After being installed, a GC column that is appropriate to the intended application is installed into the oven of the GC. Figure 4.7 is a generalized schematic diagram of a conventional GC employing a packed column. This schematic might be one that appeared over 30 years ago during the era of packed column GC. Nevertheless, this schematic serves as a good starting

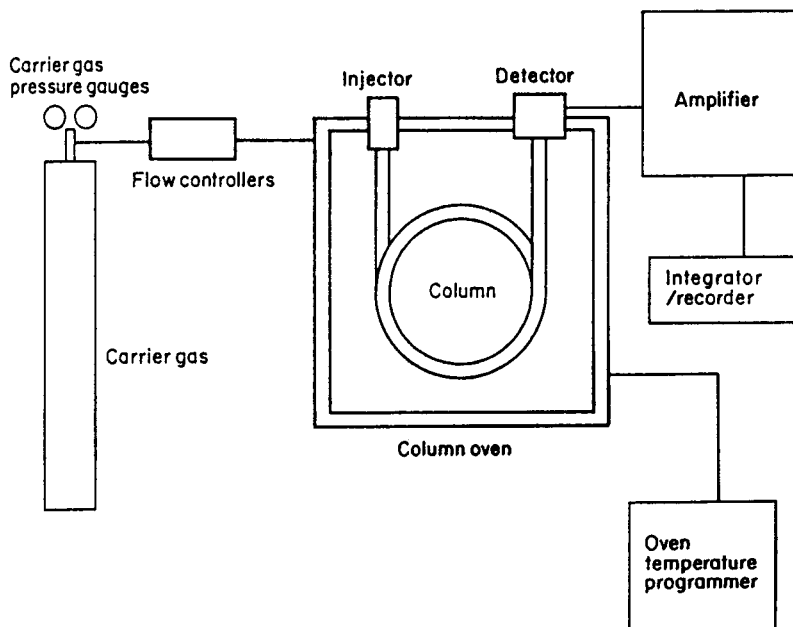
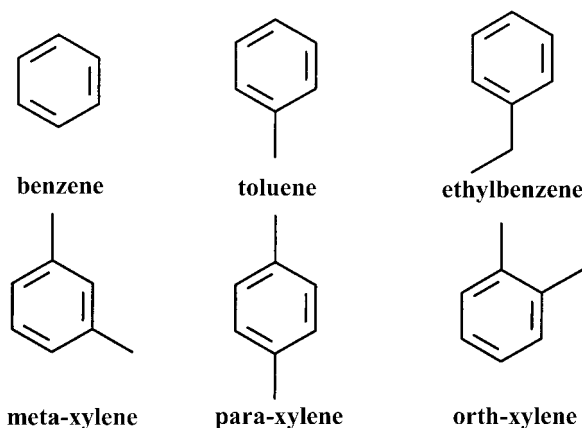


FIGURE 4.7 Schematic of a gas chromatograph.

point. We will answer the question posed above by proceeding from left to right across the diagram in Fig. 4.7 and point out how each of these instrument components have evolved since the first commercial GC appeared in 1954. Details of how one operates a GC can be found in the appropriate experiment in Chapter 5. Before we proceed to a description of a gas chromatograph, let us view the outcome of injecting a sample into such an instrument.

Figure 4.8 is an actual GC chromatogram and shows the importance of a properly installed and optimized GC as a determinative technique to organics TEQA. A groundwater sample that has been in contact with gasoline, perhaps from a leaking underground storage tank, was placed in a 22-mL headspace vial and sealed. The temperature of the vial was brought to 65°C and kept at that temperature for approximately $\frac{1}{2}$ h. The headspace was sampled using a gas-tight syringe and injected into an Autosystem GC, manufactured by Perkin-Elmer Corporation. The abbreviation, HS-C-GC-FID, refers to headspace-capillary column-gas chromatograph-flame ionization detection. BTEX refers to benzene, toluene, ethyl benzene, and meta-, para-, and ortho-xylene. These molecular structures are given as follows:



Note that the order of elution in the GC chromatogram of Fig. 4.8 is from lower to higher molecular weight. In other words, the capacity factor, k' , for benzene is much smaller than for *ortho*-xylene. From Eq. (4.31), this suggests that *ortho*-xylene has a much larger partition constant, K , into the column stationary phase in comparison to benzene. Values of k' can also be correlated with increases in boiling point for these substituted aromatics. This particular open tubular column lacks the necessary selectivity, α , to separate the *meta*- from the *para*-xylene isomer. Also note that resolution is more than adequate for all BTEX compounds except for ethylbenzene from

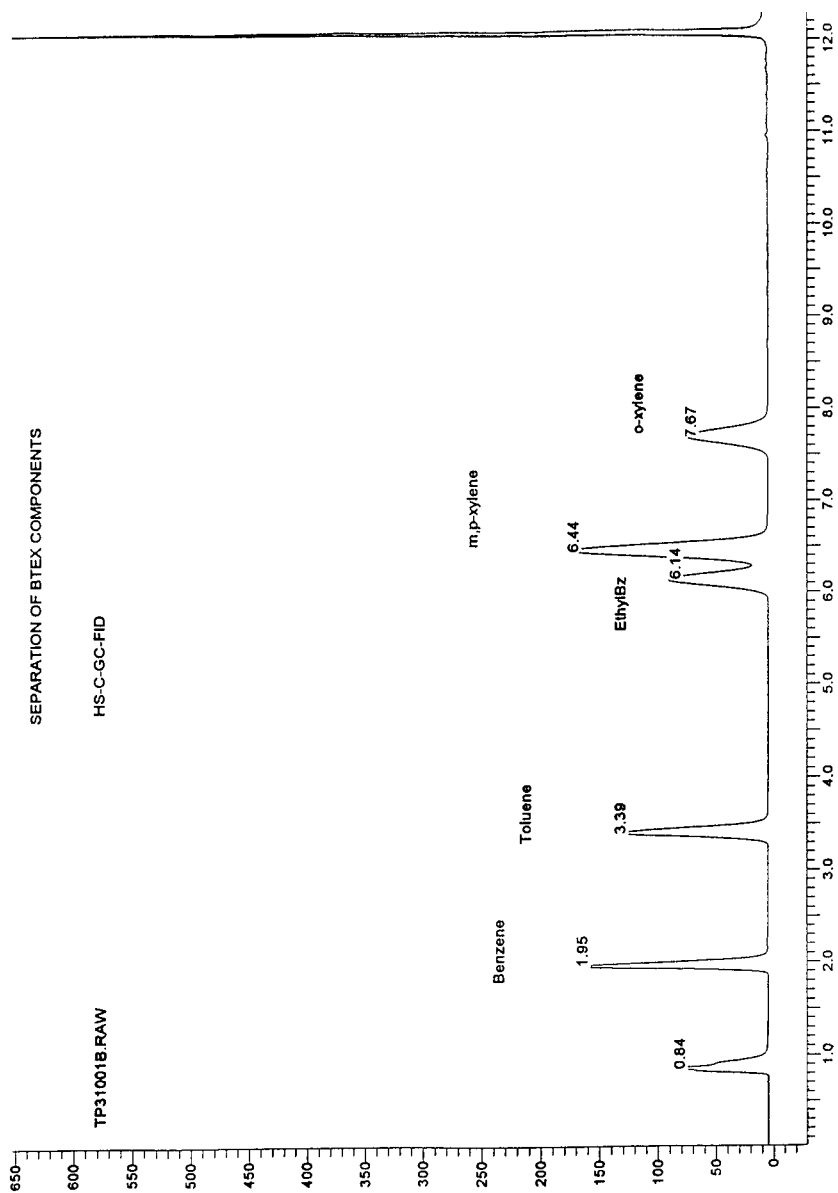


FIGURE 4.8 Separation and detection of BTEX components using static headspace capillary GC with FID.

m,p-xylene. This chromatogram was acquired by sending the analog signal from the FID to an analog-to-digital interface. The digitized data was processed by Turbochrom software, developed by PE-Nelson. We now return to our journey through Fig. 4.7

20. WHAT ARE EXTERNAL GAS PNEUMATICS AND ARE THEY IMPORTANT IN GC?

Any chromatographic separation is such that matter is forced to become more ordered. In other words, its entropy is reduced and is against the spontaneous tendency of matter to spread out. This requires an input of energy, and this input in the case of GC is provided by the potential energy of a compressed gas. Today, the chromatographer has two options with respect to carrier gas: compressed gas cylinders or gas generators. Gas generators require a large investment; however, the return on that investment is the elimination of gas cylinder handling. The annual catalogs of chromatography suppliers such as Supelco and Alltech are good sources to learn about gas generators. Carrier gas under pressure enters the GC and provides the necessary mobile phase to enable either a gas-liquid or a gas-solid chromatographic separation to occur. Let us assume that compressed gas cylinders are used as the source of carrier gas. A two-stage regulator is necessary to control the gas pressure delivered to the instrument from the source. Figure 4.9 depicts a typical compressed gas cylinder with a two-stage gas pressure regulator. For GC, the most commonly used carrier gases are helium, nitrogen, and hydrogen. Hydrogen is much more commonly found as a GC carrier gas in Europe than in the United States due to the significantly higher cost of helium. Hydrogen is also potentially flammable, unlike the other two. Most compressed gas cylinders are factory filled and, upon turning on the cylinder valve, should give a primary-stage pressure gauge reading of between 2200 and 2500 psi (pounds per square inch). The secondary-stage gas delivery pressure is adjusted from 0 to between 20 and 60 psi by turning the adjusting screw. A constant and reproducible flow rate is essential to be able to reproduce chromatographic retention times, t_R . McNair and Miller state “a compound’s retention time is a useful qualitative tool . . . two or more compounds may have the same t_R , however, no compound may have two different values for t_R ” (28). External gas pneumatics in GC consist of the following:

- Source of carrier gas and regulation of delivery pressure
- All tubing, fittings, and valves
- All gas-purifying traps

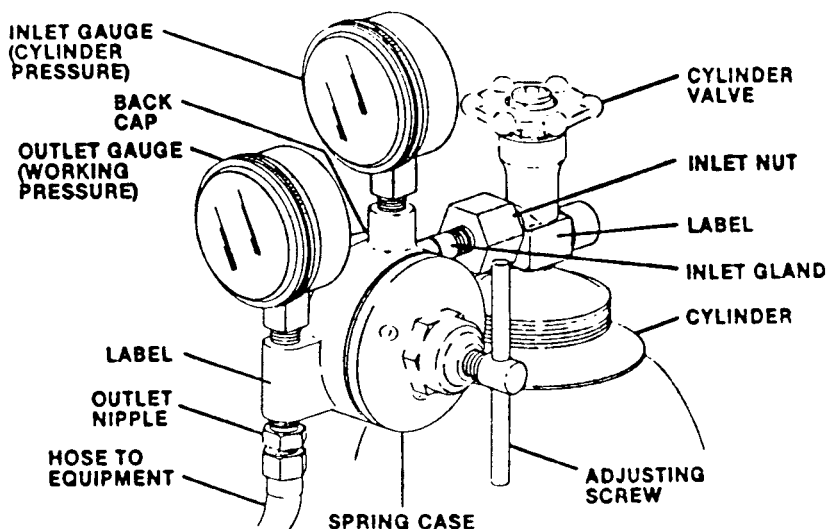


FIGURE 4.9 Representation of a typical two-stage compressed gas regulator.

All external gas pneumatics must be provided by the laboratory prior to installation of the gas chromatograph. Laboratories vary from simple external gas pneumatics, in which a single set of compressed gas cylinders is interfaced to a single GC, to a more complex configuration, in which a single set of cylinders is interfaced to two or more GCs. External gas pneumatics are, in general, essentially the same over the past four decades since compressed gas cylinders were employed. The next question to ask is how pure shall the carrier gas be that is to be delivered to the GC? The early GCs all employed a $\frac{1}{4}$ in. packed GC column and operated isothermally (i.e., the column temperature remained constant throughout the chromatographic run time). GC detectors such as the thermal conductivity detector, TCD, in those days were much less sensitive than those in use today. Gas purity was not as critical as it is today. Two options are available with respect to providing very high purity to the GC:

1. Purchase the higher-purity gas, 99.9999 % research or ultrapure grade, at significantly high cost
2. Purchase a high-purity grade, 99.995 %; only, and purchase and install relatively inexpensive traps between the tank and the GC

Most labs choose option 2 and install a series of traps that are located external to the GC unless there is some compelling reason to go with a ultrahigh- or research-grade purity carrier gas. A hydrocarbon trap is first

in line. This trap removes traces of saturated and unsaturated hydrocarbons that might be present due to residual oil from regulator diaphragms. Next should come a molecular sieve trap to remove water vapor followed by an oxygen trap. The oxygen trap is a proprietary material designed to remove traces of oxygen from the carrier gas. The presence of oxygen in the carrier gas is detrimental to GC liquid phases. Some detectors such as the electron-capture detector (ECD) respond to oxygen in the carrier gas and thus limit instrument sensitivity and, as you know from discussions in Chapter 2, this limits IDLs and MDLs.

21. WHAT ABOUT GC INLETS? WHAT DO I NEED TO KNOW?

Flow controllers are the next item in our journey through the schematic in Fig. 4.7. In the early days, before GCs controlled carrier gas flow, rotameters were installed between the regulator and the inlet to the instrument. Today, GCs themselves have pressure gauges and very fine-needle valves with which to control carrier, makeup, and detector gas flow rates. Contemporary GCs provide computerized control of both the column head pressure (i.e., the gas pressure at the column inlet) and column volumetric flow rate. Contemporary GCs will have internal pneumatics and additional traps. It is not readily apparent that the flow rate decreases as the column temperature is increased! The explanation for this is that the viscosity of the carrier gas increases as the column temperature is increased. A differential flow controller is used to provide a constant mass flow rate by increasing the column head pressure. Electronic pressure control is a relatively recent advance particularly for split/splitless GC injectors that require a constant pressure. An electronic sensor is used to detect the decrease in flow rate and cause an increase in the pressure to the column and, hence, to maintain a constant flow rate. The chromatographer needs to know the volumetric flow rate of an operating GC. When problems arise, the first question to ask is “What is the GC flow rate and is it optimized?” A word about pressure settings is in order. The setting at the second stage should be generally at least 10–20 psi higher than the setting at the inlet to the GC. This is particularly important if the sequence of gas-purification traps is used, as discussed earlier. The GC was once a single thermostatic unit. Contemporary GCs isolate the injector from the oven and from the detector. The temperature of each is set independent of the other. Injection ports that are usually operated isothermally can be temperature programmed in the same manner as GC ovens. A GC injector provides the means to introduce the liquid extract (semivolatile or non-volatile organics) or a volume of gas or headspace (gases or volatile

organics) to the chromatographic column. We discussed in detail the inlet interface to the GC for volatile organic compounds (VOC) analysis in Chapter 3. Injectors have evolved from just a heated cylindrical device that was inserted into a heater block to the split/splitless injection ports used in more contemporary GCs. The objective of injection in GC is as follows:

1. To rapidly vaporize the liquid such as an extract that contains the dissolved analyte of interest to TEQA
2. To introduce all or a portion of the vapor into a GC column
3. To split a portion of the vapor out of the injector to the atmosphere for narrow- and wide-bore capillary columns
4. To continuously clean the septum via a purge flow with carrier gas

Packed column injection ports that readily accepted a $\frac{1}{4}$ -in.-outer diameter (o.d.) stainless-steel or glass column during the early period are now are designed to accommodate $\frac{1}{8}$ -in. injection o.d. stainless-steel columns. This injection port can readily accommodate a 0.53-mm “mega-bore” (a term introduced by J & W Scientific) capillary provided that an adapter is used. Mega-bore capillary columns do not require any splitting. This enables packed column injection ports to easily accommodate mega-bore capillary columns. Contemporary GCs are dual-injector and dual-detector instruments. One injector can accommodate a $\frac{1}{8}$ -in. packed column that can be adapted to a mega-bore capillary column while the other injector can accommodate a “split/splitless” narrow- or wide-bore capillary column. The split/splitless injector requires that only a portion of the total sample volume be allowed to enter the narrow- or wide-bore capillary column. The fraction of sample that escapes to the atmosphere versus the fraction that actually enters the capillary column is determined by the split ratio. Details are given for the Autosystem[®] (Perkin-Elmer) injectors in Chapter 5.

22. WHAT IS A SPLIT RATIO AND WHAT DOES “SPLITLESS” REALLY MEAN?

The split ratio is defined as

$$\text{Split ratio} = \frac{\text{Flow rate (atmosphere)}}{\text{Flow rate (column)}}$$

Figure 4.10 shows a schematic of a split/splitless injector and visually shows the difference between the two. By measuring the carrier gas flow rate coming out of the vent and the carrier gas flow rate passing through the capillary column (be sure to turn off the makeup gas to the detector so that an accurate measurement of the column flow rate can be made), the split ratio can be calculated. As with optimum flow rate, the chromatographer should know at all times what the split ratio is and whether or not the injection was made via split or splitless. As Fig. 4.10 reveals, a splitless injection is accomplished by keeping the vent closed for t seconds after injecting an extract. McNair and Miller have recommended that splitless injection be preferred over split injection for performing TEQA. These authors clearly discuss the limitations of the splitless technique (29). Perry states that injection without splitting produces narrower bands when compared to injection with splitting due to the "solvent effect." Perry explains this phenomenon by quoting the Grobs as follows (30):

The vapourized material is transferred on to the column essentially as a mixture. In the first stage of separation, the solvent shifts away

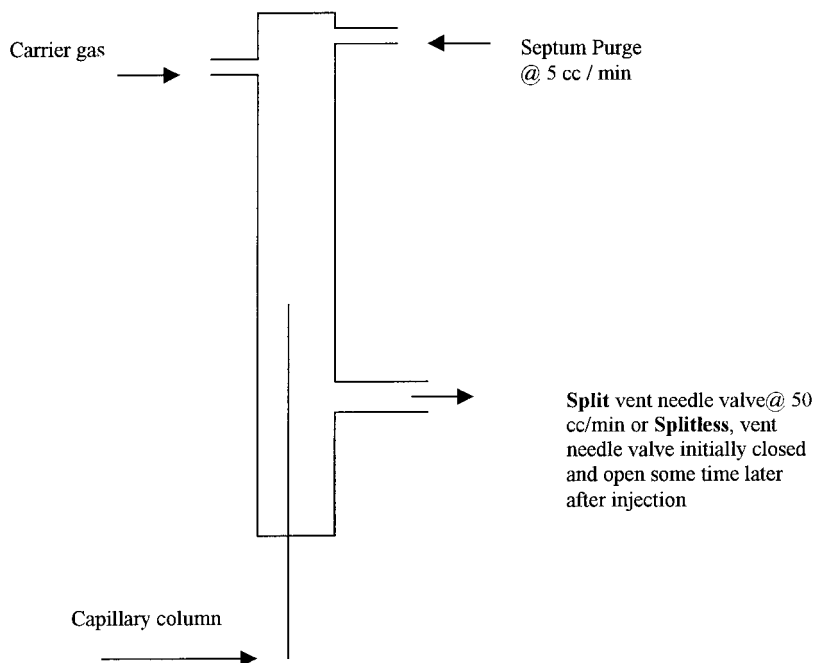


FIGURE 4.10 Schematic of a split/splitless capillary GC column injector.

from the sample components, leaving them on the back slope of its large peak. Thus, the moving vapour plugs of the sample components meet a liquid phase mixed with retained solvent, whereby the concentration of solvent increases rapidly in the direction of migration. Therefore, the front of every plug, in contact with stationary liquid containing more solvent, undergoes much stronger retention than the back of the plug. This effect causes the originally very broad bands of sample components to be condensed to a band width which, under properly selected conditions, may become even smaller than that which can be obtained with stream splitting.

Jennings has argued that splitless injection is not really without split and is a term only to be used when comparing the technique to that of split injection (31). More contemporary GCs enable the user to program a splitless injector (i.e., control of the time that a split vent remains closed). One other concept needs to be addressed before we move to the GC column in our journey through a gas chromatograph. That concept is that of sample size injected. If we are not careful, we might “overload the GC column.”

23. WHAT DOES IT MEAN TO “OVERLOAD” A CHROMATOGRAPHIC COLUMN?

One of the experiments in Chapter 5 asks the student to devise a way to increase the amount of analyte injected and to observe what happens to chromatographic peak shape. Increasing the amount of analyte to be injected in either GC or HPLC can be accomplished in one of two ways:

1. Inject identical volumes of a series of reference standards of increasing concentration
2. Inject increasing volumes of one reference standard

The result of adding too great an amount of analyte is for the observed peak shape to change from a purely Gaussian peak shape to a seriously tailed peak. Figure 4.11 illustrates this loss of peak symmetry. The amount of analyte that can be injected regardless of peak shape is limited by the linear dynamic range, R_L , of a given detector. Increasing the split ratio also helps to maintain good peak shape. Peak distortion is a column problem, whereas R_L is GC detector dependent. Perry has developed a relationship between the concentration, $C_{\max}$, corrected retention volume, V'_R , and the weight of analyte injected, w_i , according to (32)

$$C_{\max} = \frac{\sqrt{N}}{V'_R} w_i \sqrt{2\pi}$$

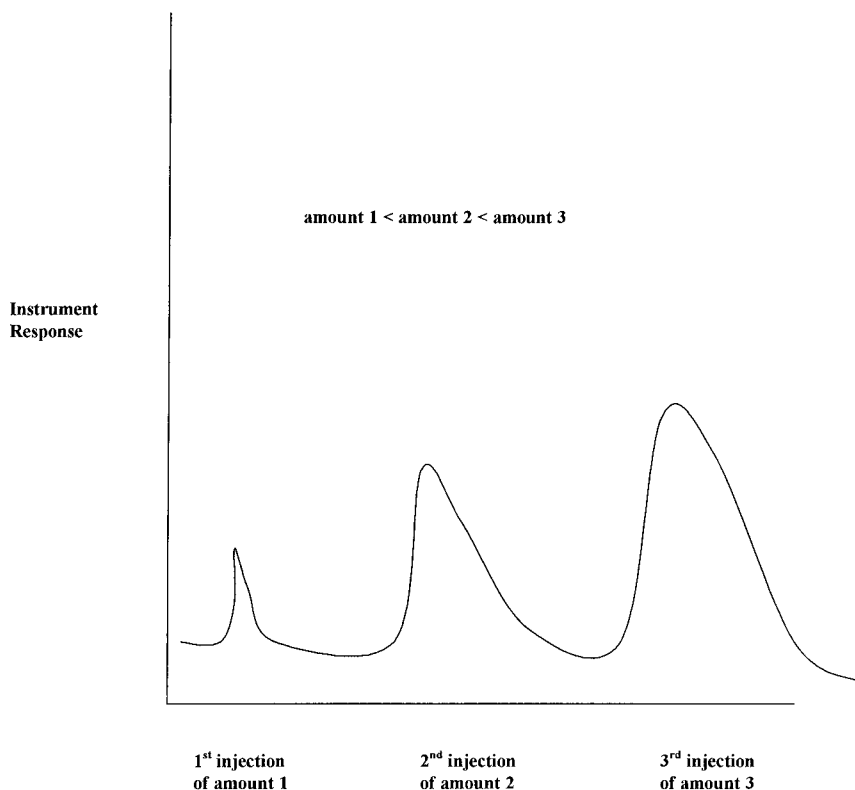


FIGURE 4.11 Typical chromatogram showing the effect of increasing the amount injected versus chromatographic peak shape.

Using this equation, Perry has shown that if w_D is the maximum weight of a given analyte with an adjusted retention time t'_R that is injected into a GC whose column contains N theoretical plates has a detector sensitivity, S , and a linear dynamic range, R_L , then

$$w_D = 0.4 \frac{SR_L t_R}{\sqrt{N}}$$

For example, assume that we are using a GC with a FID. Let us assume that the FID used has a detector sensitivity $S = 1 \times 10^{-12}$ g/s and a linear dynamic range $R_L = 1 \times 10^7$ and a column with 10,000 plates. For an analyte of interest whose adjusted retention time is 500 s, we find

$$w_D = 0.4 \frac{(1 \times 10^{-12})(10^7 \text{ g/s})(5 \times 10^2 \text{ s})}{\sqrt{1 \times 10^4}} = 2 \times 10^{-5} \text{ g}$$

This equation applies only to isothermal operation. In TEQA, our interests tend to be directed toward the lower end of R_L , although it is good to be aware of such limitations on the high end. We now continue on in our journey through a GC by moving on to what many chromatographers call the “heart of the chromatograph,” the GC column.

24. WHAT IS SO IMPORTANT ABOUT GC COLUMNS?

As in a human, the heart is the most important organ in the body! The reasons for this rest in the field of human physiology. So too, in a GC, the chromatographic column is where separations take place and the purpose of doing GC is to separate components in a mixture! Historically, packed GC columns dominated this important determinative technique. Historically, packed GC columns dominated gas chromatographic separations up until around 1980. At that time, any work with capillary columns was accomplished using glass. This required that the ends of the coiled and brittle glass columns be heated until softened to enable an installation into the GC to occur. It took a significant level of patience and skill to install a glass capillary GC column at that time. Open tubular columns owe their origins to Marcel Golay (33) and their present robustness from the development of fused-silica capillaries at Hewlett-Packard (34).

25. WHAT GC COLUMNS ARE USED IN TEQA?

The significantly large values of N for open tubular columns when compared to packed columns have established capillary columns as essential for TEQA. For example, a 60-m capillary with 3000–5000 plates/m yields 180,000–300,000 plates, whereas a 2-m packed column with 2000 plates/meter yields only 4000 plates (35). This difference in column efficiency was largely ignored by industry and regulatory government agencies during the 1959–1980 era, until the patent for open tubular columns expired. It was not until the introduction of the 0.53-mm “mega-bore” column by J & W Scientific that the EPA “allowed” the abandonment of the 6 ft $\times$ $\frac{1}{4}$ -in. o.d. packed glass column for the determination of organochlorine pesticides (OCs) via EPA Method 608! This author even heard it said in those days that “you could not connect a cap column to an ECD.” The EPA was slow to abandon the packed column mixed liquid phase consisting of 1.5%; OV-17 and 1.95%; OV 210 coated on Chromosorb. This stationary phase served

the regulatory agency very well when all that was needed was to measure trace residues for some dozen OCs. Three GC chromatograms from the earlier use of a packed column to separate and detect OCs are shown in Fig. 4.12. During the packed columns days, where N was limited, column selectivity, α , took on much more importance and numerous liquid phases became available. The open tubular column with its very large value of N does not require as large a value for α . For this reason, the number of capillary columns with different stationary phases is much more limited. In fact, it can be argued that, today, only two different cap columns are needed to handle almost all of the volatile and semivolatile priority pollutant organic analytes. McNair and Miller present the basics of the stationary phase, packed columns, and capillary columns and this source of an equivalent should be consulted for these topics (36). Narrow-, wide-, and megabore cap columns are commonplace in environmental testing labs today. A wall-coated open tubular (WCOT) column such as a DB-624 (J & W Scientific) or equivalent is usually a good first choice for trace volatile organics analysis, whereas a WCOT such as DB-5 (J & W Scientific) is a good first choice for trace semivolatile organics analysis. Perusal of GC consumable supply houses such as Alltech and Supelco are good starting points. Reviewing the appropriate EPA methods is also good practice in developing the understanding of applying GC to environment-related problems. Whether one is interested in broad-spectrum environmental monitoring or target specific analysis is also an important consideration. To understand which columns to use for a given application, Farwell has considered four basic criteria (37):

1. Polarity of analytes to be gas chromatographed
2. Polarity of the cap column stationary phase
3. Selectivity of the cap column stationary phase
4. Stationary phase stability

A knowledge of the basics of organic chemistry is very helpful in satisfying the first criterion. A scale of some kind to satisfy the second criterion would be most helpful. To satisfy the third criterion requires contributions from both the analyte and stationary phase, and criterion 4 refers to the physical attributes of the stationary phase. Figure 4.1 serves as a reminder of the limited scope of gas chromatography. Organic compounds of environmental interest must be vaporizable and, at the same time, be thermal stable at injector temperatures of 200–300°C. These two criteria are met only for those analytes that are neutral, relatively nonpolar consisting of aliphatic and aromatic carbon classes. As oxygen begins to get incorporated into the molecule, the ability of a molecule to meet these two criteria becomes more limited. Consider this series of aromatics, starting with benzene, then mov-

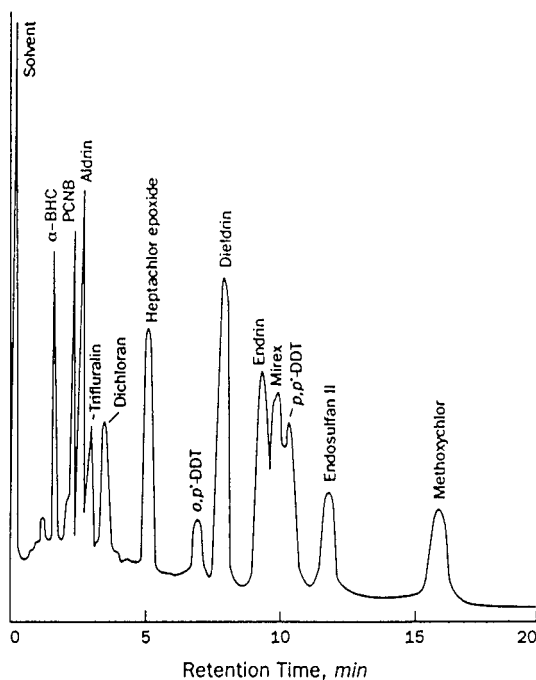
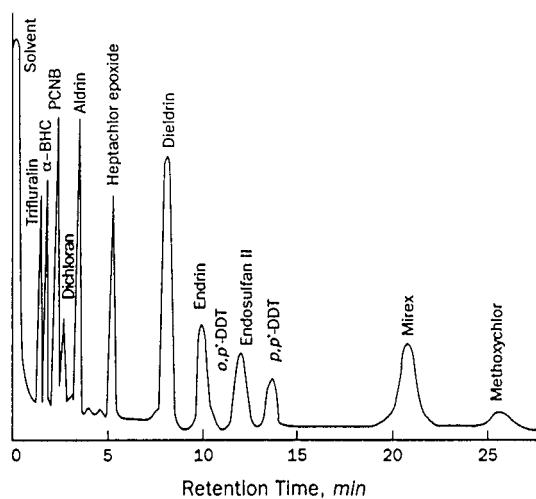


FIGURE 4.12 Comparison of chromatograms for the separation and detection of priority pollutant organochlorine pesticides for three different packed GC columns.

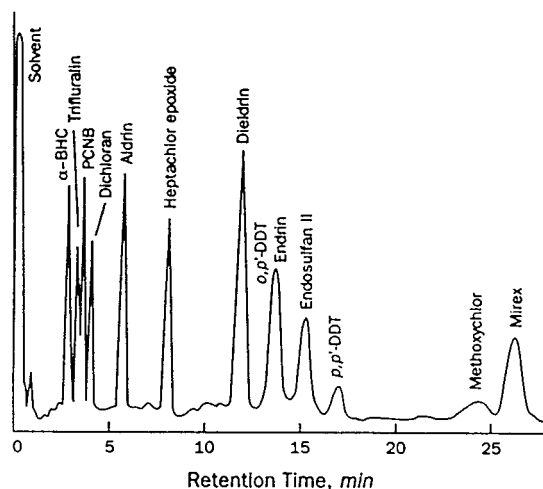
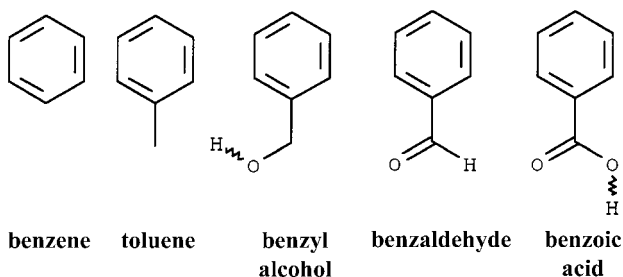


FIGURE 4.12 (continued)

ing to toluene by substituting a methyl group for hydrogen. We then replace a hydrogen in the methyl group with a hydroxy group and get benzyl alcohol. We proceed to partially oxidize this benzyl group to an aldehydic group and obtain benzaldehyde. Finally, we fully oxidize the aldehydic functional group to a carboxylic group and obtain benzoic acid. The structures are as follows:



The first four monoaromatics in this series are liquids at room temperature, and benzoic acid is a solid. It is possible to gas chromatograph benzoic acid; however, peak distortion and lowered sensitivity result. The polarity of these compounds takes us from the volatile, nonpolar benzene out to the semivolatile, polar benzoic acid. Benzoic acid can be moved back across the domain to the more volatile and nonpolar region

by conversion of this carboxylic acid to its methyl ester by chemical derivatization techniques. A more exact definition of solute polarity was developed by Kovats, who stated that “polarity cannot be expressed with one simple parameter” (38).

26. WHAT IS THE KOVATS RETENTION INDEX?

The Kovats Retention Index for a specific organic compound (i.e., the analyte of interest on a specific GC column) is nothing more than a relative retention time! The index is in relation to aliphatic hydrocarbons. For example, the hydrocarbon iso-octane, or 2,2,4-trimethylpentane if gas chromatographed, would probably have a retention time between that of the straight-chained alkanes, *n*-octane C₈ and *n*-nonane C₉. The Kovats Index for *n*-octane is 800 and that for *n*-nonane is 900. Iso-octane would then have a Kovats Index somewhere in between, such as 860. The Kovats Index is calculated from a GC chromatogram using the retention volume for the analyte whose index is sought, V_R^{unknown} , and the retention volumes for the alkane with *n* carbon atoms per molecule, V_R^n , and for the alkane with *n* + 1 carbon atoms per molecule, V_R^{n+1} , using the following relationship:

$$I = \left[\frac{\log V_R^{\text{unknown}} - \log V_R^n}{\log V_R^{n+1} - \log V_R^n} \right] + 100n \quad (4.33)$$

Note that the Kovats Retention Index is independent of the chemical nature of the column. If we were to chromatograph the five mono-aromatics cited above on a purely nonpolar stationary phase such as Squalane (a C₃₀ aliphatic hydrocarbon), we would generate a series of *I* values for each compound. If we then chromatograph these same compounds on a more polar stationary phase such as diethyleneglycolsuccinate (DEGS), we would obtain an entirely different set of *I* values. We calculate the difference in Kovats Indices as follows:

$$\Delta I^{\text{benzene}} = I_{\text{DEGS}}^{\text{benzene}} - I_{\text{Squalane}}^{\text{benzene}}$$

$$\Delta I^{\text{benzylalcohol}} = I_{\text{DEGS}}^{\text{benzylalcohol}} - I_{\text{Squalane}}^{\text{benzylalcohol}}$$

Thus, stationary phases can have their polarities compared for a given test “probe” such as benzene or benzyl alcohol. In an attempt to reduce the number of liquid phases used in packed column GC, McReynolds published

an extensive listing of Kovats Retention Indices using 10 “probes” for hundreds of liquid phases at that time (39). Rohrschneider considered only five probes, namely, benzene, ethanol, 2-butanone or MEK, nitromethane, and pyridine (40). McReynolds expanded this to 10 probes and proved his assumption that liquid phases had, indeed, proliferated because many liquid phases possessed “McReynolds constants” that differed little (41). The reason for choosing which organic compounds are suitable probes is outlined in Table 4.7 (42). Usually only the first five probes are cited. Characterizations of GC liquid phases is an ongoing research area. Carr and co-workers have developed a new set of GC-based solute parameters using solvatochromic linear solvation energy relationships (43). McNair and Miller considered a thermodynamic view of the interaction between a solute and a stationary phase and devised a definition of the separation factor or selectivity α between solutes A and B according to (44)

$$\alpha = \frac{K_C^B}{K_C^A} = \frac{p_A^\circ \gamma_A}{p_B^\circ \gamma_B}$$

where p° is the vapor pressure of the solute as if it were a pure liquid and γ is the activity coefficient for a specific solute. This equation shows that the extent of separation between solutes A and B depends not only the ratio of the vapor pressure of the pure solutes but also on the ratio of their activity coefficients. This explains why two analytes with very similar boiling points (i.e., similar pure solute vapor pressures, p°) have significantly different retention times.

27. WHY DON'T I SEE McREYNOLDS/ROHRSCHEIDER CONSTANTS IN CURRENT CHROMATOGRAPHY CATALOGS?

A perusal of a current catalog of one the largest suppliers of chromatography and related products in the United States makes minimal mention of McReynolds constants and no mention of Rohrschneider constants. Perusal of the current catalog of a second large supplier of chromatography and related products does not even mention these constants! Perhaps this reflects the commercial maturity of GC, in that GC has been around for some 40 years and applications have become well known. The popularity of open tubular columns have reduced the need for numerous liquid phases; hence, there is little need to distinguish liquid phases anymore. The most commonly used liquid phases for TEQA are the family of silicone polymers (also called polysiloxanes). As introduced in Chapter 1, the EPA organics protocol is

TABLE 4.7 McReynolds Probes and the Rationale for Its Selection

Symbol	Test compound	Basic molecular interactions	Functional group
X'	Benzene	Dispersion with some weak proton-acceptor properties	Aromatics, olefins
Y'	Butanol	Orientation properties with both proton-donor and proton-acceptor capabilities	Alcohols, nitriles, acids
Z'	2-Pentanone	Orientation properties with proton-acceptor capabilities	Ketone, ethers, aldehydes, esters, epoxides, dimethylamino derivatives
U'	Nitropropane	Dipole orientation properties	Nitro and nitrile derivatives
S''	Pyridine	Weak dipole orientation with strong proton-acceptor capability	Aromatic bases
H'	2-Methyl-2-pentanol		Branched alcohols
L'	1,4-dioxane		
M'	Cis-Hydrindane		

divided into volatile organic compounds (VOCs) and semivolatile to non-volatile organic compounds (SVOCs). Either a 30 or 60-m $\times$ 0.53 or 0.75-mm cap column with a stationary phase specifically designed for the separation of VOCs is commonly used. The chemical nature of these phases are considered proprietary bonded phases by most suppliers. For example, a 75-m $\times$ 0.53-mm i.d. with a 3- μ m film thickness is sold by Supelco as their SPB-624, VOCOL cap column to separate some 93 VOCs without subambient temperatures in less than 20 min! The 624 designation refers to the EPA method number as applied to wastewater analysis. The 105-m $\times$ 0.53-mm i.d. with a 3- μ m film thickness developed in the early eighties by Restek (called their Rtx-502.2 column) is based on a diphenyl/dimethyl polysiloxane which provides low bleed and thermal stability up to 270°C. The 502.2 designation refers to the EPA method number as applied to drinking water analysis. SVOCs are commonly separated by using a WCOT column that contains a 5% phenylmethyl/dimethyl polysiloxane. The column length for adequately separating SVOCs is generally 30 m. Narrow-bore and wide-bore columns are used with a split/splitless injector, and a mega-bore cap column is installed on an $\frac{1}{8}$ -in. packed column injector without split. SVOCs can be further subclassified into the 100 or so priority pollutant base/neutral and acids (BNAs), 30 or more organochlorine pesticides (OCs), polychlorinated biphenyls (PCBs), and a miscellaneous category for many other analytes of environmental concern. VOCs can be subclassified as polychlorinated ethylenes and ethanes (ClVOCs), monoaromatics from fuel contamination of groundwater (BTEX), trihalomethanes from chlorine disinfection of drinking water (THMs), and a miscellaneous category for many other analytes of environmental concern. This classification scheme is shown in a flowchart in Fig. 4.13. This author believes that a good silicone WCOT, if properly maintained and if appropriate sample prep techniques are always used, will last for years! Two other types of cap columns used in GC are support-coated open tubular (SCOT) and porous-layer open tubular (PLOT). SCOT columns are no longer widely used, whereas PLOT columns are essential for the separation of permanent gases (i.e., Ne, Ar, O₂, N₂, CO₂, Kr, Xe, and lower-molecular-weight aliphatic hydrocarbons). Let us consider these fused-silica WCOTs in more detail.

28. WHAT ARE FUSED-SILICA POLYSILOXANE-COATED WCOTs ANYWAY?

One of this author's earliest laboratory experiences involved placing a piece of glass tubing into a burner flame and observing the softening of the center portion of the tubing. At some point in the heating, upon pulling on both

TABLE 4.8 Most Commonly Used Liquid Phases in Capillary GC Along with Their McReynolds Constants

Liquid phase	Commercially available name	Range of operating WCOT temperatures	Range of McReynolds constants
Dimethyl polysiloxanes (gum, oil)	DB-1, HP-1, BP-1, OV-1, OV-101, SE-30, SP-2100	-60 to 280 (oil) -60 to 325 (gum)	220-229
5% Phenyl methylpolysiloxane	DB-5, BP-5, SPB-5, SE-52, OV-73, RT _x -5, HP-5	-60 to 325	334-337
50% Phenyl methylpolysiloxane	DB-17, HP-17, OV-17, GC-17, SP-2250	40 to 280	884
50% Trifluoropropyl methylpolysiloxane	DB-210, OV-210, QF-1, SP-2401	40 to 240	1520-1550
25% Cyanopropylmethyl 25% phenylmethylpolysiloxane	DB-225, OV-225, SP-2300		
Poly(ethylene glycol) gum	DB-WAX, HP-20M, SP-1000, Carbowax 20M	50 to 220	2301-2309

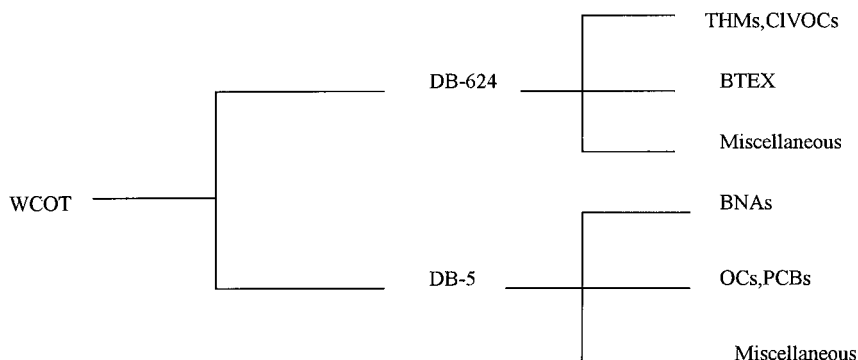


FIGURE 4.13 Flowchart for choosing the appropriate WCOT column.

ends of the unheated portions of the tubing, fine capillary tubing can be produced. This is still the principle of cap column fabrication. Figure 4.14 depicts a capillary drawing machine and some detail of the actual coating of the outside sheath (45). These schematics are based on the original design of Desty et al. (46). A good discussion of the fabrication and technical challenges of polysiloxane WCOTs has been written by one of the founders of such cap columns (47). One of the more detailed reviews of liquid phases can be found in a series of three articles by Yancey (48). Polysiloxanes are chemically bonded to the fused silica using proprietary surface treatment techniques. Jennings has summarized these approaches as follows (49):

Peroxides may be added to the coating solution, and static coating procedures are generally employed to deposit a film of vinyl-containing stationary phase oligomers on the interior wall of the tubing. Heating causes peroxide decomposition, which yields free radicals and initiates cross-linking (and, if the surface has been properly prepared, surface bonding) when the column is heated. The preferred peroxide is generally dicumyl peroxide, which decomposes to form volatile products that are dissipated during the heating step.

Let us focus on the WCOT columns used to implement EPA Method 507 “Determination of Nitrogen- and Phosphorus-Containing Pesticides in Water by GC with a Nitrogen-Phosphorous Detector”. The method is summarized as follows (50):

A measured volume of drinking water of approximately 1 L is extracted with methylene chloride by shaking in a separatory funnel

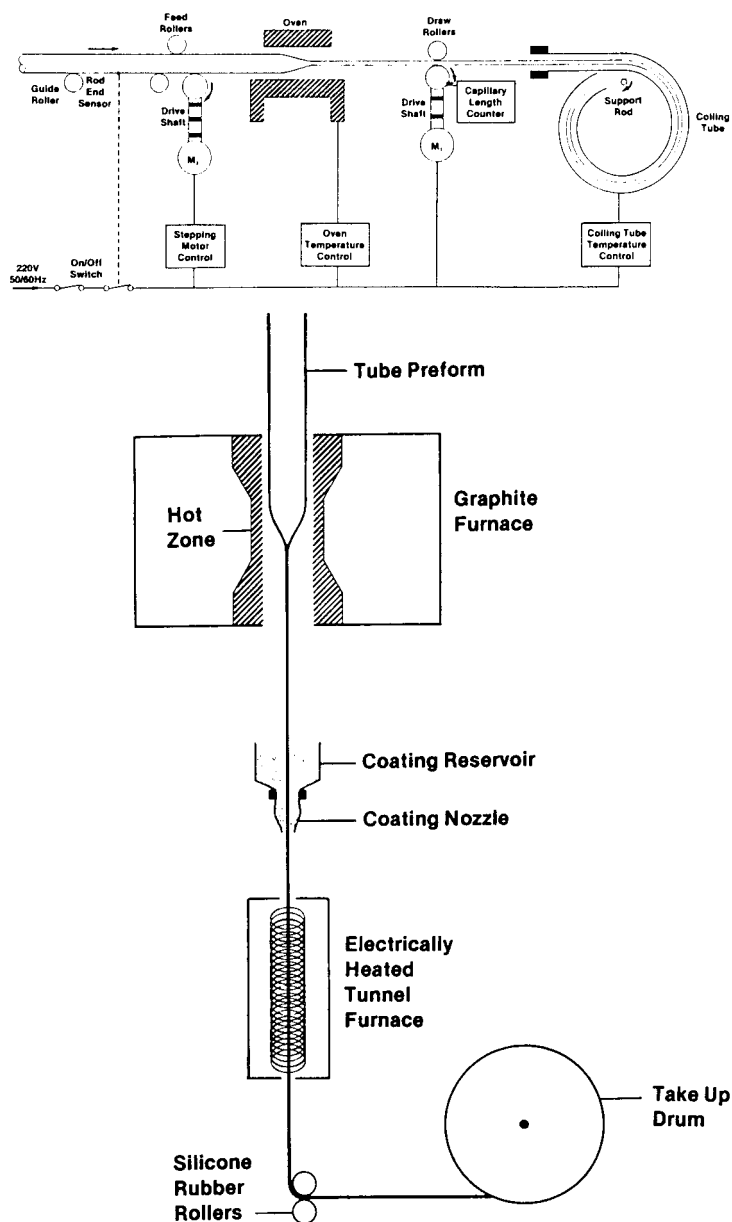


FIGURE 4.14 Schematic diagram of the apparatus used for drawing fused-silica capillary GC columns. (From Ref. 45.)

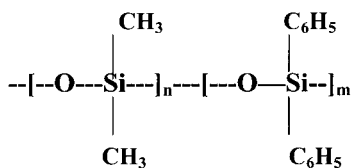
or mechanical tumbling in a bottle. The methylene chloride extract is isolated, dried and concentrated to a volume of 5 mL during a solvent exchange to methyl *tert*-butyl ether, MTBE. Chromatographic conditions are described that permit the separation and measurement of the analytes in the extract by capillary column GC with a nitrogen–phosphorous detector, NPD.

A primary WCOT is required to separate and detect N/P containing pesticides that may be present in drinking water. The separated N/P pesticides must be confirmed by injecting the MTBE extract into a confirmatory WCOT. The primary WCOT is DB-5 from J & W Scientific or equivalent; the confirmatory WCOT is DB-1701 from J & W Scientific or equivalent. The molecular structure for each of these polysiloxanes are shown in Fig. 4.15. The liquid phases are similar in the n repeating monomer, whereas they differ in the substitution in the m repeating monomer unit. The DB-5 is a 5% phenyl/dimethylpolysiloxane and the DB-1701 is a 14% cyano-propyl/phenyl/dimethylpolysiloxane. The introduction of a cyano group increases the polarity of this WCOT and, hence, shifts the retention time for the N/P pesticides that are to be monitored. A methyl *tert*-butyl ether (MTBE) extract from an unknown drinking water sample if injected onto both column will give yield values for t_R that differ by a fixed number of minutes. These retention times would have been previously determined by injecting standards onto both columns. The only other alternative to dual WCOT identification and confirmation is to use single WCOT and both an element specific detector such as a nitrogen–phosphorus detector (NPD) and a mass spectrometric detector. Table 4.9 is a sample from the list of some 46 individual N/P pesticides/herbicides that are to be monitored in drinking water by this method. Alachlor is a substituted acetanilide herbicide in widespread use today and atrazine and simazine are examples of triazine herbicides also in widespread use. Dichlorovos and hexazinone are illustrative of organophosphorus pesticides. Triphenyl phosphate is used as an internal standard in the method. Note the 1–2-min or so shift in the N/P pesticide/herbicide.

The differences in capacity factors among different polysiloxane WCOT columns is made quite evident in Fig. 4.16. In this figure, two GC chromatograms are “stacked” using Turbochrom (PE-Nelson) software and obtained on gas chromatographs in the author’s laboratory. A 100-ppm reference standard consisting of AR 1260 dissolved in acetone is taken and 1 μ L of this solution is injected into a GC that contains a DB-5 (J & W Scientific) to yield the top chromatogram, and the same solution is injected into a GC that contains a DB-1 (J & W Scientific) to yield the bottom chromatogram. The liquid-phase DB-1 is a dimethylpolysiloxane.

Diphenyldimethylpolysiloxane (e.g. DB-5)

- non-polar
- excellent general purpose column
- wide range of applications
- low bleed
- high temperature limit
- bonded and cross-linked
- solvent rinsable
- wide range of column dimensions available
- equivalent to USP Phase G27

Cyanopropylphenylmethyldipolysiloxane (e.g. DB 1301,1701)

- low to mid polarity
- bonded and cross-linked
- solvent rinsable
- equivalent to USP Phase G43

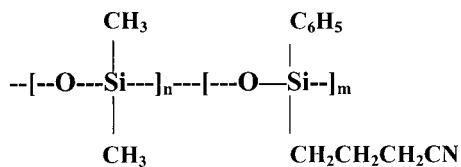


FIGURE 4.15 Two different liquid phases used in capillary GC. (From J & W Scientific Inc. 1998 Catalog.)

TABLE 4.9 Representative N/P Pesticides/Herbicides, Their Classification, and Retention Times on Two Chemically Different GC Columns

N/P pesticide	Classification	$t(R)$ on DB-5 (min)	$t(R)$ DB-1701 (min)
Alachlor	Substituted acetanilide herbicide	35.96	34.10
Atrazine	Triazine herbicide	31.77	31.23
Dichlorvos	Organophosphorus pesticide	16.54	15.35
Hexazinone	Organophosphorus pesticide	46.58	47.80
Simazine	Triazine herbicide	31.49	31.32
Triphenyl phosphate	Internal standard	47.00	45.40

If methyl groups are substituted for the phenyl groups in the DB-5 structure shown in Fig. 4.15, a DB-1 structure is obtained. Commercially available equivalents to DB-1 include HP-1, HP-101, Ultra-1, SPB-1, Rtx-1, OV-1, SP-2100, and SE-30 among others. The influence of the phenyl groups in DB-5 creates additional aromatic group intermolecular interactions that influence the partitioning constant and, hence, according to Eq. (4.31), the capacity factor, k' . Use of these two WCOT columns provides acceptable identification and confirmation for this complex mixture of polychlorinated biphenyl congeners. Two other significant concepts with respect to WCOTs and their use in TEQA are film thickness, d_f , and the influence of column temperature on k' .

29. WHY IS WCOT FILM THICKNESS IMPORTANT?

Because it influences the C term in the Golay equation! The Golay equation as applied to WCOT is similar to that developed earlier [e.g. Eq. (4.25)] and is by:

$$H = \frac{B}{\bar{u}} + (C_M + C_S)\bar{u} \quad (4.34)$$

Note the absence of the A term and the separate resistance to mass transfer in the mobile phase, C_M , and in the stationary phase, C_S . This equation can be written even more specifically and we have

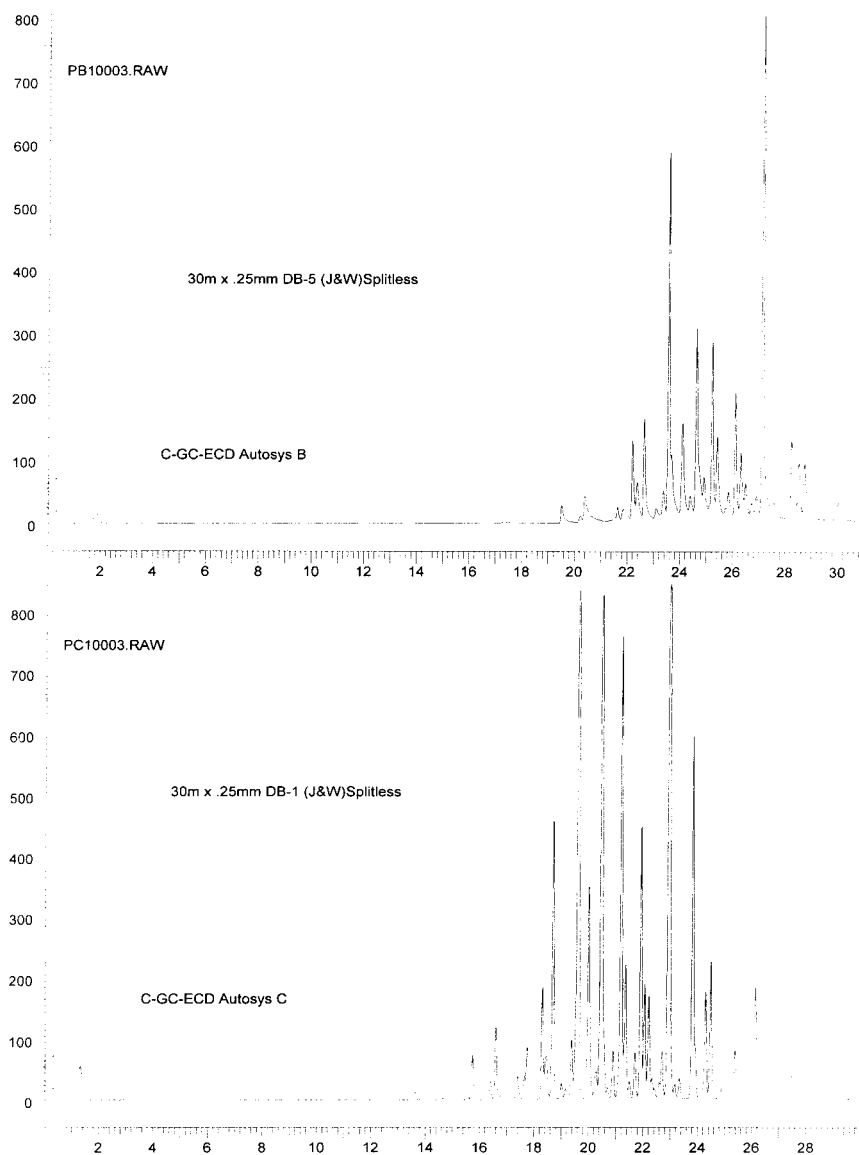


FIGURE 4.16 Comparison of the GC chromatograms upon injecting a Aroclor into a DB-5 versus a DB-1 WCOT.

$$H = \frac{2D_M}{\bar{u}} + \left(\frac{(1 + 6k' + 11k'^2)}{24(1 + k')^2} \right) \left(\frac{r_C^2}{D_M} \right) \bar{u} + \left(\frac{2k'}{3(1 + k')^2} \right) \left(\frac{d_f^2}{D_S} \right) \bar{u} \quad (4.35)$$

The Golay equation shows a dependence of H on the square of d_f in the C_S term. While we are discussing this equation, note the dependence of H on the square of the column radius, r_C , in the C_M term. The choice of GC carrier gas through the WCOT influences the diffusion of analyte in the mobile-phase, D_M , and the diffusion of analyte into the stationary phase, D_S . Figure 4.17 is a Golay plot of H versus the average linear carrier gas velocity (in cm/s) for nitrogen, helium, and hydrogen. A WCOT with a 25-m $\times$ 0.25-mm i.d. and $d_f = 0.4 \mu\text{m}$ thickness. Nitrogen as the carrier gas yields the lowest optimized H , whereas the lighter gases, He and H_2 , do not have as severe a resistance to the mass transfer term and can be operated at much higher linear velocity. Figure 4.17 should always be kept in mind when considering which gas to use in GC.

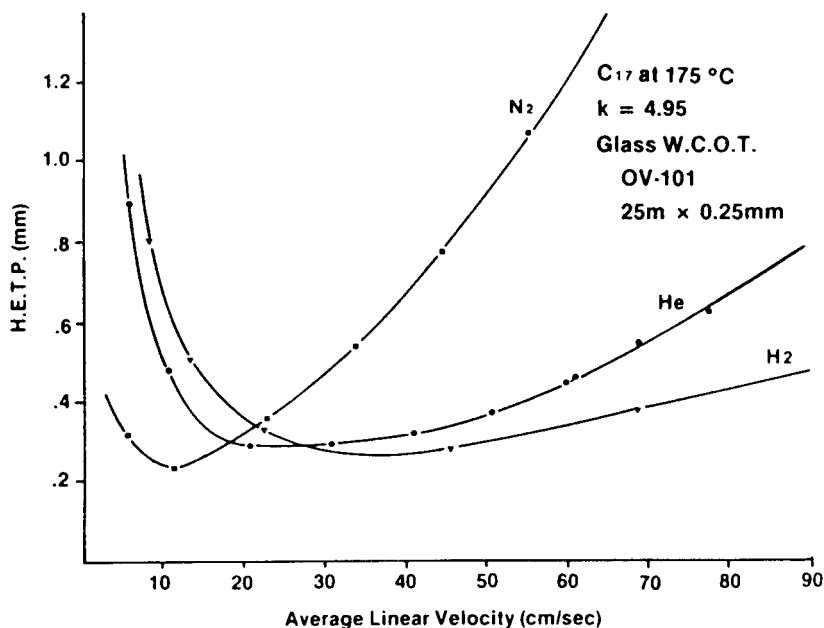
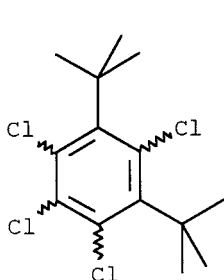
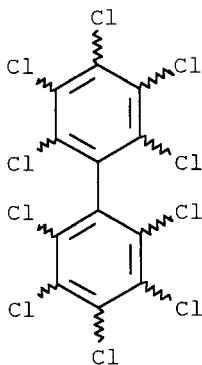


FIGURE 4.17 Effect of carrier gas on van Deemter curve, 0.25 mm i.d. WCOT, $d_f = 0.4 \mu\text{m}$.

The B term, the contribution to H due to longitudinal diffusion, becomes evident as the two chromatograms are compared in Fig. 4.18. The top GC chromatogram shows an optimized carrier gas flow rate and a separation of the two surrogate compounds required for the determination of various OCs and PCBs in such methods as EPA Method 8080. The molecular structures of both surrogates are as follows:



**Tetra-chloro-
meta-xylene**



Decachlorobiphenyl

These highly chlorinated aromatics have k' values that serve to bracket the k' values for the GC elution of all OCs and PCBs. The bottom chromatogram in Fig. 4.18 is at approximately half the volumetric flow rate of the top chromatogram. Both peaks are tailing while their retention times have increased somewhat. A much stronger ECD signal is also evident. These surrogate compounds are so significantly different in their k' 's that it would require a much more elevated column temperature to elute DCBP. A fully chlorinated biphenyl is as nonpolar and nonvolatile as one can get! In fact, as Fig. 4.18 demonstrates, it requires keeping the column temperature at 275°C. This is the recommended maximum operating temperature of the WCOT to finally elute DCBP from the column! Column temperature can significantly influence the magnitude of an analyte capacity factor, k' . All broad-spectrum monitoring EPA methods using WCOTs use GC with a column temperature that varies with time after sample injection.

30. HOW DOES COLUMN TEMPERATURE INFLUENCE k' ?

We know that the retention time of any organic compound is influenced by the column length, the linear velocity of the carrier gas, and the magnitude

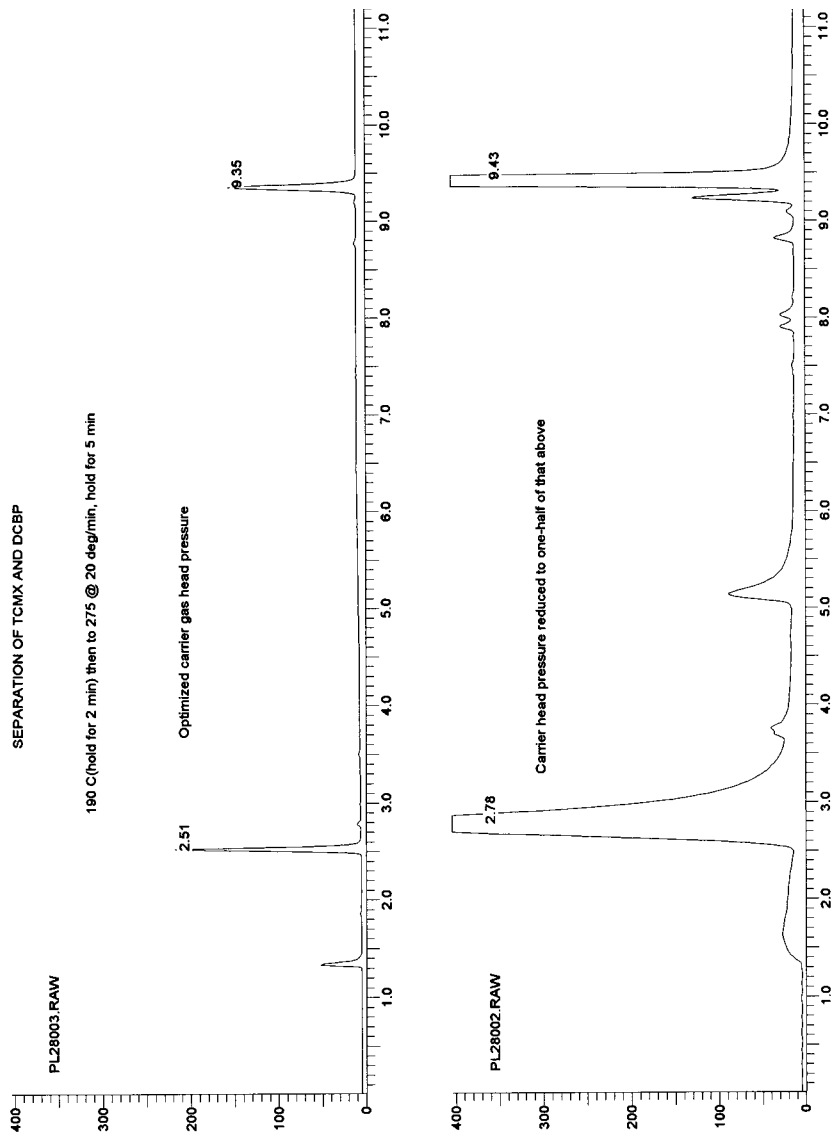


FIGURE 4.18 Comparison of GC chromatograms under optimized carrier gas head pressure and reduced head pressure conditions.

of the compound's capacity factor, k' . Revisiting Eq. (4.17), rewriting it, and solving for t_R , we get

$$t_R = t_0[1 + k'] = \frac{L}{u}(1 + k')$$

In Eq. (4.10), a chromatographic capacity factor, k' , was defined in terms of the product of a partition constant and a phase ratio denoted by β . For a WCOT, β is related to film thickness, d_f , and column diameter, d_C , according to (51)

$$\beta = \frac{d_C}{4d_f}$$

For example, a 250- μm i.d. WCOT column with a film thickness of 0.2 μm has a $\beta = 313$, whereas a 320- μm WCOT column with $d_f = 3.0 \mu\text{m}$ has a $\beta = 26.7$. Recall that $k' = K/\beta$, and if K , the partition constant for a compound between the mobile and stationary phases, is held constant, an increase in d_f reduces β while increasing k' and, of course, t_R . If d_C is increased, for the same film thickness, d_f , then a higher β results. This higher phase ratio for a fixed value of the partition constant, K , serves to decrease k' and result in a shorter retention time. In general, higher β values such as $\beta > 100$ are more suitable for SVOCs, whereas lower β values such as $\beta < 100$ are better for VOCs (51). Once a WCOT column is chosen, only the flow rate and column temperature can be varied so as to maximize chromatographic resolution, R_S . We have discussed the influence of flow rate on R_S earlier and what remains is to discuss the effect of column temperature, T_c , on the separation factor, α , and on R_S . Figure 4.19 shows how both k' and R_S depend on T_c . Injection of a solution that contains these four aliphatic hydrocarbons at a $T_c = 45^\circ\text{C}$ yields the top chromatogram shown in Fig. 4.19. The peaks are all adequately resolved; however, the run time is excessive, over 40 min! Increasing the column temperature to 75°C reduces chromatographic run time but also decreases chromatographic resolution. A further increase to a $T_c = 95^\circ\text{C}$ further decreases R_S to unacceptable levels. The optimum T_c that maximizes R_S for all pairs of closely spaced peaks would be somewhere between 45°C and 75°C . Note the change in elution order as peak 1 is retained longer than peak 2.

If the $\log K$ is plotted against the reciprocal of absolute temperature, $1/T$ for a given organic compound, a straight line is established. The slope of the line varies somewhat with the nature of the solute. At certain tem-

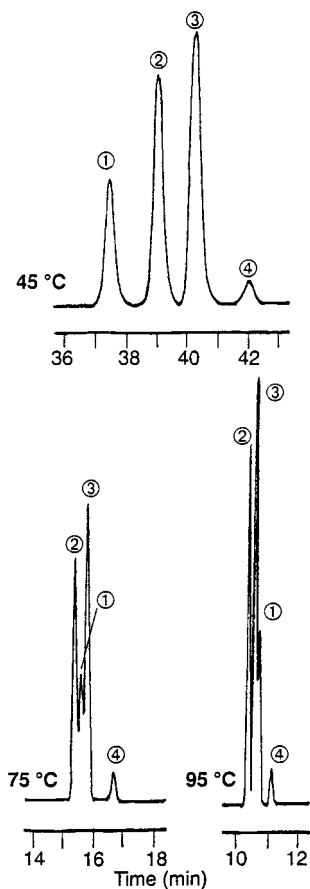


FIGURE 4.19 Separation of four hydrocarbons at different temperatures. 45-m $\times$ 0.5-mm-i.d. support-coated open tubular column coated with squalane. Peak identification: (1) methylcyclohexane, (2) 2,5-dimethylhexane, (3) 2,4-dimethylhexane, and (4) 2,2,3-trimethylpentane.

peratures, the lines can cross and this explains the observed reversal in elution order as shown in Fig. 4.19. Such a plot is shown in Fig. 4.20. At $T_c = T_A$, peak 1 elutes before peak 2, whereas at $T_c = T_B$, peak 2 elutes before peak 1. Differences in the slope are caused by the difference in analyte – stationary phase interactions. From Eq. (4.30), we know that R_S depends on the separation factor, α , so that for a pair of peaks, it is of interest to know just how α varies with increasing the WCOT column temperature.

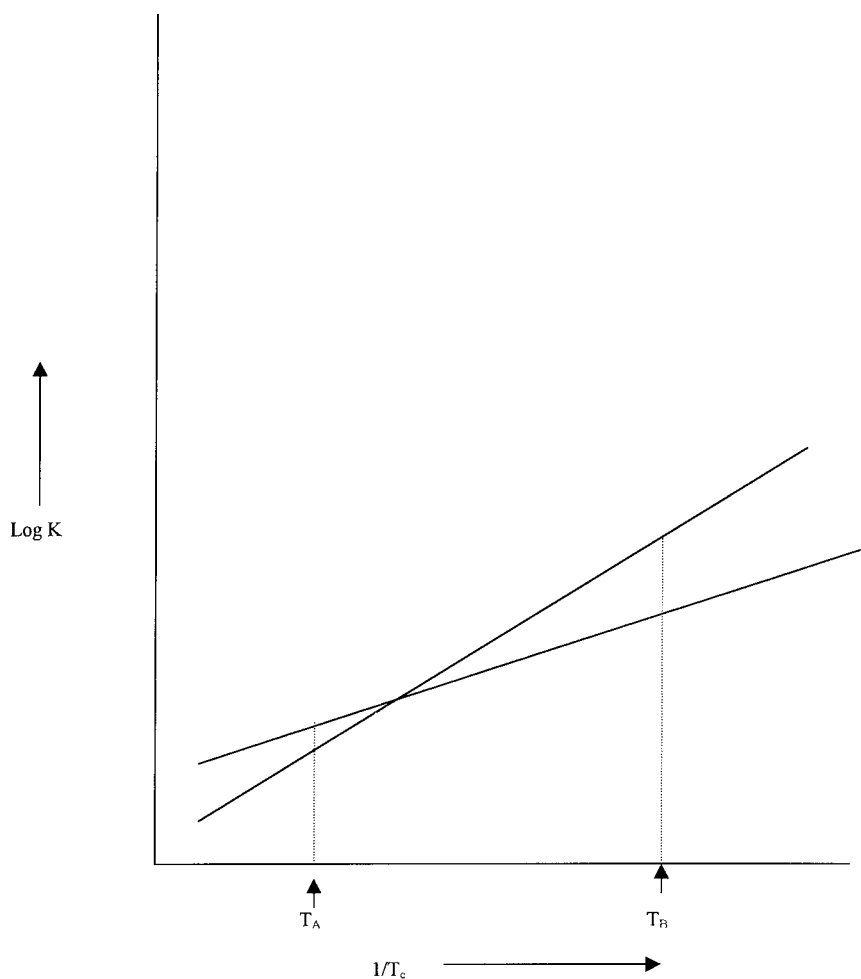


FIGURE 4.20 Plot of the logarithm of an analyte's chromatographic distribution constant between mobile and stationary phases and column temperature for two different chemical compounds.

The Clausius–Clapeyron equation in physical chemistry states

$$\log p^\circ = -\frac{\Delta H_v}{2.3RT} + C$$

where p° is the vapor pressure exerted by a substance if it were pure at a absolute temperature T . R is the ideal gas constant, C is a constant, and Δ

H_v is the change in the substance's enthalpy in moving from a liquid to a vapor and is also called the heat of vaporization. If T_c is increased, the following happens (52):

1. k' and t_R both decrease.
2. α can either rise, peak out then fall, or fall, bottom out, then rise or steadily decrease (53).
3. N slightly increases.

In the case of our two surrogates discussed earlier (TCMX and DCBP), their respective p° (alternatively, their respective boiling points) values differ by such a wide range that if we set the T_c to a fixed value (e.g., 225°C), we would be able to elute TCMX but not DCBP! If the GC is capable, increasing the T_c after injection of the sample assures that both surrogates will be eluted within a reasonable chromatographic run time as was done in Fig. 4.19. One says then that the way to elute both surrogates is to "temperature program." For this reason, programmed-temperature gas chromatography (PTGC) is one of the most powerful features of contemporary GC instrument design. According to Perry (54):

PTGC also brought about direct control of carrier gas flow rate. Before PTGC, carrier gas flow was adjusted by pressure control. However, because gas viscosity and therefore the column pressure drop, ΔP , increases with increasing T_c , the carrier gas flow tends to decrease during PTGC. Such a decrease is quickly reflected in the deviating baseline signal from a single, flow-sensitive detector. To hold the carrier gas flow constant during PTGC, and thus also the retention times (from run to run) and particularly the baseline (during each run), flow controllers were introduced. They became standard, expected components in gas chromatographs . . . the prime characteristics of both GCs and stationary phases became those relevant to the demands of programmed temperature. The GC had to be able to bring about sharp but well-controlled changes in column temperature, and yet record a gas chromatogram; the stationary phases had to withstand these changes without decomposing or vaporizing to a prohibitive degree, and yet retain the selectivity relevant to each . . . to permit rapid changes in column temperature, high-velocity, high-power, low-mass column ovens were introduced. PTGC or no, the column oven must allow easy column installation and interchange; therefore air had to remain the

heat-exchange medium. In the newer designs, however, the air would be moved in much greater quantity and velocity, by and over much more powerful fans and heaters. Further, the column oven would become low-mass. A light inner aluminum shell of low thermal mass, insulated from the more substantial walls of the outer oven.

31. WHAT IS PTGC ANYWAY?

Just after a sample is introduced by either manual or autosampler syringe into the GC injection port, the oven temperature (and hence the column temperature) is increased via a previously programmed series of increases in T_c at a constant rate of change (i.e., dT_c/dt is fixed), along with periods of time where T_c does not change. These periods of time are called holds or isothermal steps. GCs can be temperature programmed either in one step or in a series of steps, each step having a different value for the ramp or, as defined earlier, dT_c/dt . Each step can be viewed as consisting of an initial temperature with its hold time, a ramp at a fixed value, and a final temperature with its own hold time. Contemporary GCs are temperature programmed via commercially available chromatography processing software with a precision to 0.02 min or less! This aspect of the GC oven has advanced considerably over the past 40 years to accommodate PTGC. Earlier instruments used clock-motor-driven potentiometers to change the column oven setpoint at a constant rate. Instruments of the early 1980s vintage had electronic temperature programmers that used plug-in resistors to set one or more programming rates. This author's experience with "thumbwheel settings" was obtained using the Model 3700 (Varian Associates). As a rule of thumb, retention times decrease by about one-half for each 15–20°C increase in T_c (55). McNair and Miller have pointed out that for a homologous series, analyte retention times are logarithmic, whereas under isothermal conditions, analyte t_R values are linear when temperature programmed (56). Onuska and Karasek have pointed out in their classic text on environmental analysis that the Kovats Retention Index, [Eq. (4.33)] can be replaced with the following relationship to give a linear scale (57):

$$I^{\text{PTGC}} = 100 \left(\frac{t_R^x - t_R^n}{t_R^{n+1} - t_R^n} \right) + 100n$$

32. CAN WE FIND HOW RETENTION TIME VARIES WITH T_c ?

Yes, Perry has considered the relationship between a solute's specific retention volume, V_g (the net retention volume, V_N , per gram of liquid phase) and the column temperature T_c (58). Perry's approach is not seen in most other GC texts, so let us derive it here. Before we present his approach, we need to shore up some fundamentals first. One of the few chromatographic relationships to really commit to memory is the relationship among solute retention volume, V_R , column void volume, V_M , and the solute chromatographic capacity factor, k' , according to

$$V_R = V_M(1 + k') \quad (4.36)$$

The net retention volume, V_N , is defined as the difference between the solute retention volume and the void volume according to

$$V_N \equiv V_R - V_M = k' V_M = \left(\frac{K}{\beta}\right) V_M$$

Upon eliminating the phase ratio, β , we get

$$V_N = K \left(\frac{V_L}{V_M}\right) V_M = K V_L$$

where V_L is the volume of the liquid phase. This is another of those important chromatographic relationships that help in understanding all of this. If you double the volume of the liquid phase, you can expect a doubling of the solute net retention volume. Perry has shown earlier that V_N can be related to V_g . He defines V_g as the net retention volume per gram of stationary phase of density ρ_L and volume V_L in the column with the temperature, T_c , of the column corrected to 0°C in the following way:

$$V_N = \left(\frac{T_c}{273}\right) V_L \rho_L V_g$$

Eliminating V_N between these two equations gives

$$K = \left(\frac{T_c}{273}\right) \rho_L V_g$$

Taking the common logarithms of both sides of this equation yields

$$\log K = \log V_g + \log \frac{\rho_L T_c}{273} \quad (4.37)$$

The Clausius–Clapeyron equation introduced earlier can be differentiated with respect to temperature, and the temperature term itself manipulated to yield

$$\frac{d}{d(1/T)}(\ln p^\circ) = -\frac{\Delta H_v}{R}$$

An analogous statement for the temperature rate of change of the Henry's Law constant, K_H , discussed in Chapter 3, can be found in texts on chemical thermodynamics. The differential molar heat of vaporization of a solute from an infinitely dilute solution, ΔH_S , can be related to the Henry's Law constant according to:

$$\frac{d}{d(1/T)}(\ln K_H) = -\frac{\Delta H_S}{R}$$

Upon elimination of the term $d(1/T)$ from both of these differential equations, we can rewrite

$$\frac{d(\ln K_H)}{d(\ln p^\circ)} = \frac{\Delta H_S}{\Delta H_v} = a$$

so that

$$d(\ln K_H) = ad(\ln p^\circ)$$

Henry's Law constant is essentially the inverse of the chromatographic partition coefficient, $K = 1/K_H$ and this allows us to restate this equation as

$$d(\ln K) = -ad(\ln p^\circ) \quad (4.38)$$

Combining Eqs. (4.37) and (4.38) while eliminating $\ln K$ yields a relationship between V_g and the vapor pressure of the pure solute, p° . Laub and Pecsok have also arrived at this relationship (59):

$$\log V_g = -a \log p^\circ + \text{const} \quad (4.39)$$

Returning to the Clausius–Clapeyron equation and integrating, we obtain

$$\ln p^\circ = -\frac{\Delta H_v}{RT} + \text{const}$$

Upon multiplying through by a and converting to common logarithms, we get

$$a \log p^\circ = -a \left(\frac{\Delta H_v}{2.3RT} \right) + \text{const}$$

Upon substituting for the term “ $a \log p^\circ$ ” into Eq. (4.39) gives

$$\log V_g = - \left(\frac{-a\Delta H_v}{2.3RT} + \text{const} \right) + \text{const}$$

Because $\Delta H_S = a\Delta H_v$, we can now arrive at the ultimate objective of Perry's derivation:

$$\log V_g = \frac{\Delta H_S}{2.3RT_c} + \text{const}$$

The specific retention volume, a chromatographic property of a given solute of interest, is related to the column temperature such that plots of $\log V_g$ versus $1/T_c$ should yield a straight line whose slope is related to either the heat of vaporization of the solute at infinite dilution or, if $a = 1$ (if Raoult's Law holds), the heat of vaporization of the pure solute. Studies of column efficiency via a consideration of the effect of T_c on H ($N = H/L$) have shown that an optimum T_c can be found that minimizes H or maximizes N . The interested reader can refer to the excellent text by Harris and Hapgood on PTGC (60). Hinshaw has offered some additional insights into PTGC (61).

33. THIS IS ALL WELL AND GOOD, BUT DON'T I FIND TEMPERATURE PROGRAMS ALREADY PROVIDED IN EPA METHODS?

Yes, you do if you are implementing the broad-spectrum monitoring of these methods. For a shorter list of analytes of environmental interest, a knowledge of the effect of T_c on various GC conditions is important. Also, be aware that there is a finite amount of time needed to cool the instrument down from the maximum T_c reached in a given temperature pro-

gram. Experience in the operational aspects of PTGC using different reference standards is a great way to gain the necessary “hands-on” knowledge that will pay great dividends in any future laboratory work involving GCs. We would not know that we have achieved an optimized temperature program if it were not for the ability to detect the eluted analyte of interest to TEQA.

34. WHAT ARE THE COMMON GC DETECTORS AND HOW DO THEY WORK?

We continue now on our journey across the GC schematic as shown in Fig. 4.7 to the topic of GC detectors. An overview of the common detectors along with their commonly used abbreviations is found in Table 4.10. We will discuss only those detectors of relevance to TEQA and mention only some of the more recently developed GC detectors. This author was recently confronted with the notion of removing an existing detector from a GC and replacing it with another type. This is not as straightforward as one might think. The one universal concept here is that all GC detectors produced an analog signal related to the analyte that has just eluted the chromatographic column. This magnitude of this signal, whether positive or negative, should always be viewed with respect to the noise that is also generated by the detector and the processing electronics. The reader is referred to the topics involving the signal-to-noise ratio discussed in Chapter 2. One experiment in Chapter 5 asks the student to compare organic compound specificity for a FID versus an ECD.

The GC detector is the last major instrument component to discuss. The GC detector appears in Fig. 4.7 as the “box” to which the column outlet is connected. Evolution in GC detector technology has been as great as any other component of the gas chromatograph during the past 40 years. Among all GC detectors, the photoionization (PID), electrolytic conductivity (EICD), electron-capture (ECD), and mass selective detector (MSD) (or quadrupole mass filter) have been the most important to TEQA. The fact that an environmental contaminant can be measured in some cases down to concentration levels of parts per trillion (ppt) is a direct tribute to the success of these very sensitive GC detectors and to advances in electronic amplifier design. GC detectors manufactured during the packed column era were found to be compatible with WCOTs. In some cases, makeup gas must be introduced, such as for the ECD. Before we discuss these GC detectors and their importance to TEQA, let us list the most common commercially available GC detectors and then classify these detectors from several points of view.

Most GC detectors in use today along with the primary criteria used to compare such detectors are listed in Table 4.10. It seems that the develop-

TABLE 4.10 Summary of the Operating Characteristics of All of the Common GC Detectors Used in TEQA

Abbrev.	Selectivity	Flow dependence	Destructive or not	Detector stability	Linear dynamic range	Sensitivity	Importance to TEQA	Used by author
TCD	Universal	C	N	Good	$> 10^5$	> 10 ppm	Some	Yes
FID	Selective for C	M	D	Good	$> 10^7$	> 10 ppm	Some	Yes
PID	Selective for aromatics	C	N	Moderate	$> 10^7$	> 1 ppb	Much	Yes
EICD	Selective for X, S, N	C	D	Moderate		> 1 ppb	Much	Yes
ECD	Selective for X	C	N	Moderate	$> 10^4$	> 1 ppb	Much	Yes
TSD	Selective for N, P	M	D	Good	$> 10^7$	> 1 ppb	Some	Yes
FPD	Selective for N, P, S	C	D	Good	> 10	> 1 ppb	Some	Yes
MSD	Universal and selective	M	D	Good	$> 10^6$	> 10 ppb	Much	Yes

Note: C = concentration; N = nondestructive; M = mass; D = destructive.

ment of new GC detectors over the past 10 years is rare. This reflects more the maturity of gas chromatography as an analytical determinative technique rather than the lack of innovative ideas. We will discuss two of the more recent commercially developed GC detectors after we discuss the most common ones that have been around for 30 or more years. The following question is appropriate at this point. If you can detect down to ppt levels and can do this with a good signal-to-noise ratio, why change to a newly introduced detector? Each GC detector listed in the table can be further categorized as being either universal or selective, concentration or mass flow dependent, and destructive or nondestructive to the analyte as it elutes from the chromatographic column. Additional questions must be asked. Does the detector have a baseline that is stable over time? Over what range of concentration or mass will the detector give a linear signal? Just how sensitive is the detector or with respect to an entire instrument such as a GC, how low can it measure (the IDL, refer to Chapter 2)? Is the detector very important to TEQA? Has this author ever operated such a detector, or, in other words, can he write from experience?

Ewing has defined a concentration-dependent GC detector as one that gives a response proportional to the concentration of sample, expressed in mole fraction. He also defines a mass-dependent GC detector as one that depends upon the rate at which the sample is delivered to the sensing element, but the extent of dilution by carrier gas is irrelevant (62). The distinction between a concentration and mass-dependent GC detector has to do with understanding just what is fundamentally responsible for the signal that originates from such a detector. A Gaussian profile of a chromatographically resolved peak is shown in Fig. 4.21. The nature of the signal that is responsible for any rise in the detector response when an analyte passes through is viewed in terms of an infinitesimal area, dA . For a concentration-dependent detector such as a thermal conductivity detector (TCD) or an electron-capture detector (ECD) having a mole fraction x_S that can be defined in terms of the velocity of solute v_S and a carrier gas velocity, v_C , we can define dA as follows:

$$dA = x_S dt = \left(\frac{v_S}{v_S + v_C} \right) dt$$

Let us assume that as the analyte of interest elutes from the column into the detector, the total gas velocity can be composed of contributions from the solute as well as from the carrier gas. Mathematically stated, $v = v_S + v_C$ so that upon integration over the entire peak, we can show that

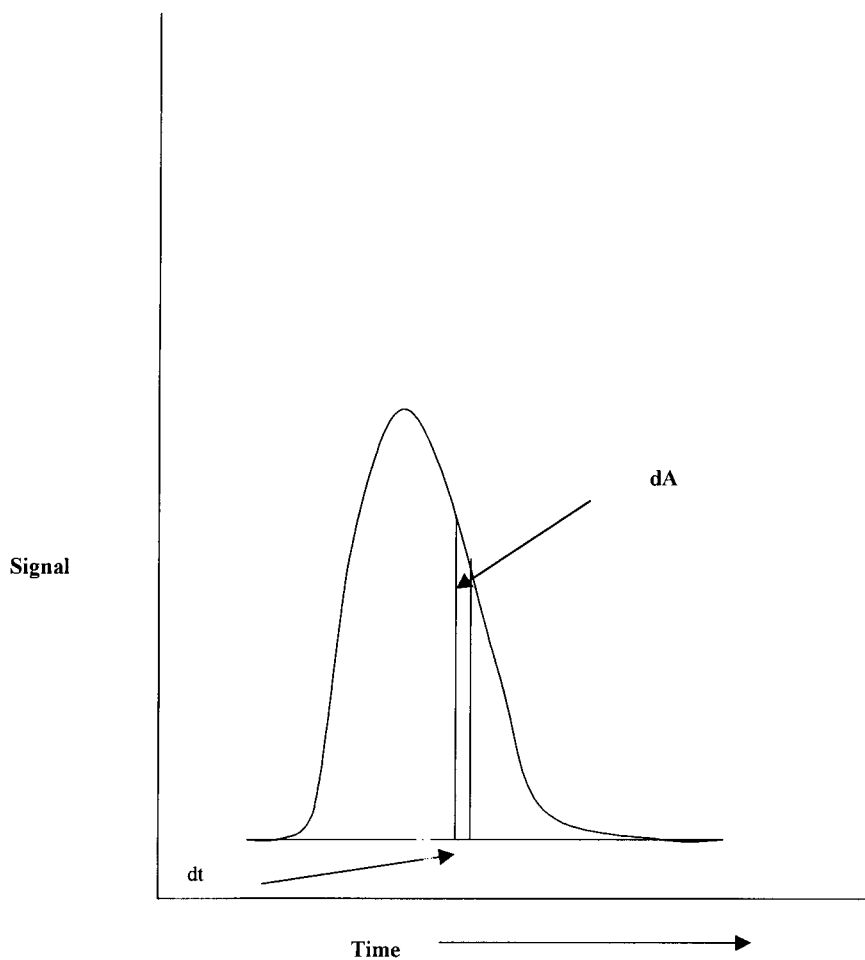


FIGURE 4.21 Profile of a Gaussian peak shape showing an infinitesimal slice of the peak area, dA .

$$A = \int x_S dt = \int \frac{v_S}{v} dt$$

The solute velocity is the time rate of change of mass, $v_S = dm/dt$, and we can express the integral as

$$A = \frac{1}{v} \int \frac{dm}{dt} dt = \frac{m_S}{v}$$

The above equation states that the peak area is proportional to the solute mass, m_S , only if v is held constant. We have thus shown that the peak area is indeed related to a ratio of solute mass to total flow rate.

Again, consider Fig. 4.21, but in this case, we have a mass-dependent GC detector, and we now define the infinitesimal strip, dA , as

$$dA = v_S dt$$

The area under the curve is obtained as

$$A = \int v_S dt = \int \frac{dm}{dt} dt = m$$

The peak area is directly related to the mass of solute passing through it. A concentration-dependent detector does not necessarily respond to the presence of a substance in the carrier gas but rather to a difference or change in the concentration of that substance. Hence, a TCD would have its sensitivity defined as a smallest number of microvolt-seconds per ppm, whereas the sensitivity of a flame ionization detector (FID) might be defined as the smallest number of microvolt-seconds per nanogram. Recall from the discussions in Chapter 2 that sensitivity refers to the magnitude of the slope of a plot of detector response versus analyte concentration or analyte mass, and the detection limit of the GC detector or as part of an instrument (IDL) refers to a signal-to-noise ratio measurement. This author believes it to be very important that practicing analysts and lab technicians have some understanding as to how GC detectors work! It helps to solve a lot of problems later! The TCD has been around for a long time. Aside from the TCD being used in environmental testing labs to measure fixed gases and any organic compounds that are present at low parts per hundred (%) levels or higher, this detector does not have a role in TEQA. The FID, however, has a somewhat limited role in TEQA largely due to a FID detection limit in the 10-ppm realm. The FID is suggested in some EPA methods for SVOCs screening procedures. The basic design of the FID is often a starting point for explaining how other GC detectors work. For these reasons, we delve into the details of this GC detector.

35. HOW DOES A FID WORK?

The FID is a micro hydrogen/oxygen burner that continuously maintains a hydrogen flame. A collector electrode is set at +300 V relative to the flame tip. When an organic compound is eluted from the GC column, it is ionized and a small current is generated. It is this current that forms the basis for

the analog signal from the FID whose magnitude is proportional to the mass of compound being detected. Carrier gas being chemically inert does not cause the formation of ions. An igniter is provided to start the hydrogen flame burning. Hence, the FID looks like a “chimney with two side arms.” One side arm is for the electrical connections for the igniter; the other side arm provides for the polarizing voltage and analog signal output. Lighting a FID is one of the more memorable analytical lab type experiences one might confront. Recently, this author, who is accustomed to checking to see whether or not the FID is lighted by holding the lens on his glasses above the outer barrel of the detector, changed his glasses from glass to plastic lenses. An accumulation of moisture on the lens surface is indicative of a lighted FID. A small area on the surface of the plastic lens, instead of accumulating water, melted, much to the surprise and chagrin of the author! Figure 4.22 shows a schematic of an FID. Carrier gas (either He, N₂, or H₂) enters from beneath, being joined by hydrogen and then air and up through cylinder to the jet tip. Above the jet tip is a collector cup/electrode arrange-

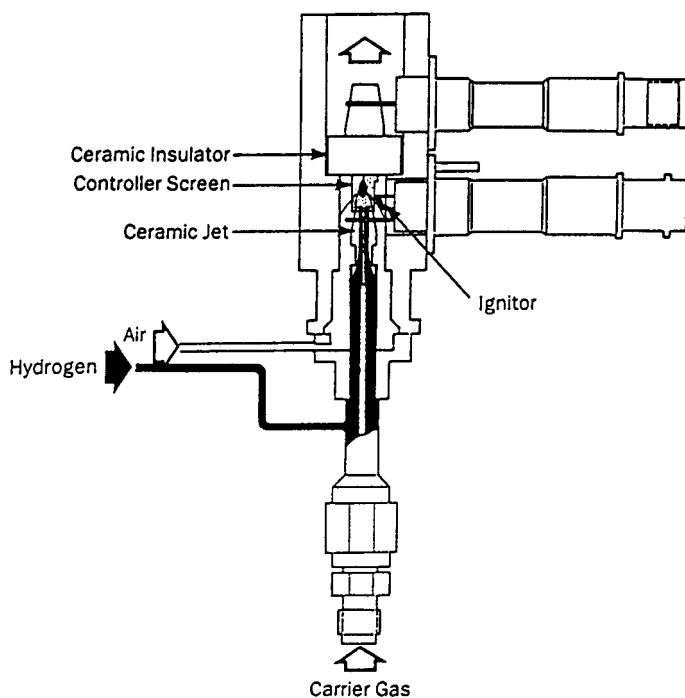


FIGURE 4.22 Schematic of a flame ionization GC detector.

ment. Coaxial cables lead out from the side of the FID and connect to instrument electronics.

The operating characteristics of the FID serve as a good frame of reference from which to view all other GC detectors. It is for this reason that we discuss these FID operating characteristics. We will then discuss the ECD, PID, EICD, TCD, FPD, and MSD in terms of how each of these compares to the FID. This author has had numerous requests over the years to use the FID to measure down to low ppb such as 10–100 ppt of an aliphatic or aromatic organic compound by a direct injection of a solution that contains the dissolved compound. This it cannot do! A gas chromatograph incorporating a cap column and a FID, abbreviated C–GC–FID, does not have an IDL of 1 ppb or lower! Prospective clients are surprised to learn this. Let us take a look at these FID operating characteristics.

36. WHAT ARE THE OPTIMUM GAS FLOW RATES AND OPERATING CHARACTERISTICS OF A FID?

Compounds that do not contain organic carbon do not burn and are insensitive to the FID. These include water, inert gases, and nonmetal hydrides such as ammonia and hydrogen sulfide. The greater the number of carbon atoms per molecule in an organic compound, the greater is the FID sensitivity. For example, injection of 1 μL of a 50-ppm solution containing *n*-hexane dissolved in acetone would yield a signal that is strong yet less than the signal from injection of 1 μL of a 50-ppm solution containing *n*-nonane dissolved in the same solvent. One says that *n*-nonane has a greater FID relative response factor (RRF) than does *n*-hexane. Aromatic hydrocarbons have a greater RRF when compared to aliphatic hydrocarbons having the same number of carbon atoms per molecule. As shown in one of the GC experiments in Chapter 5, a student can keep the carrier gas flow rate and the FID airflow rate fixed while varying the FID hydrogen flow rate and deduce that 30–50 cm^3/min for a conventional FID maximizes the GC response. Keeping the carrier and FID hydrogen flow rates fixed while varying the FID airflow rate reveals that beyond 300 cm^3/min , no further increase in GC response is observed.

McNair and Miller have characterized the FID based on the following criteria (63). The FID is selective for all carbon-containing compounds. The FID has a minimal detectable amount of 10^{-11} g. A linear dynamic range of 10^6 enables, for a 1- μL injection volume, a detector response that is linear from 1 g of hydrocarbon all the way down to 1 μg of hydrocarbon. The next criterion is that of good baseline stability over time and this translates into minimal baseline drift. Also, the effect of a change in flow rate or temperature on baseline stability is an important characteristic of FIDs. Finally, it is

important that a GC detector be thermally stable because the analyte must remain in the gas phase. The FID can be operated up to a maximum temperature setting of 400°C. The GC chromatogram shown for the separation of BTEX components in gasoline-contaminated groundwater (Fig. 4.8) was obtained using a FID. The EPA's Contract Laboratory Program utilizes a preliminary method known as "Hexadecane Screening of Water and Soil Screening for Volatiles." This method comprises a preliminary evaluation of a hazardous waste sample prior to implementing the determinative GC-MS technique in EPA Method 8270. In the author's laboratory, a C-GC-FID is a very useful instrument for conducting preliminary examinations for the presence of trace organic contaminants in drinking or wastewater samples. For example, if gasoline-contaminated drinking water is suspect, a mini-LLE (liquid-liquid extraction) using hexadecane with a subsequent injection of 1 μ L of the hexadecane extract into the C-GC-FID will give the analyst a "quick yet informative" indication of the extent of the contamination.

More than 20 years ago, the need to separate and detect VOCs was accomplished by placing a PID in series with a EICD in which aromatic VOCs could be detected on the PID and CIVOCs could be detected on the EICD. This was the principal basis for the EPA Methods 501/502 for drinking water and 601/602 for wastewater. The lowering of GC IDLs from the low-ppm level afforded by the FID to the low-ppb level was first accomplished on a routine basis with the PID, EICD, and ECD detectors. An instrument that incorporated a cap column and ECD, C-GC-ECD, first detected DDT in the environment and a cap column with a PID, C-GC-PID, first found ppb levels of BTEX in contaminated groundwater.

37. HOW DO A PID AND AN EICD WORK?

Configuring both the PID and EICD in tandem has proven to be a powerful duo for relatively clean water samples. EPA Method 8021B in the most recent SW-846 series recommends that both detectors be used in tandem or individually. The PID is an aromatics-specific detector and the EICD is a halogen-specific detector. When placed in tandem, both GC detectors provide for a large number of VOCs that can be separated and detected using WCOTs. The PID consists of a deuterium lamp that provides the necessary 10.2 eV of energy to ionize an aromatic molecule such as benzene while unable to ionize many other hydrocarbons whose ionization potential is greater than 10.2 V. A neutral molecule, RH, absorbs a photon of ultraviolet (UV) energy and dissociates into a parent ion and an electron according to



The energy of the photon must be of a frequency such that this energy exceeds the ionization potential of the organic compound for photoionization to occur. The PID is equipped with a sealed UV light source that emits photons that pass through a UV transparent window made of LiF, MgF₂, NaF, or sapphire into an ionization chamber where photons are absorbed by the eluted analyte. A positively biased high-voltage electrode accelerates the resulting ions to a collector electrode. The current produced by the ion flow is measured by the electrometer and is proportional to analyte concentration in the gas phase. The PID is a concentration-dependent, nondestructive detector. Other lamps are available that enable a wider range of hydrocarbons to be as sensitive as aromatics. A schematic of the PID is shown in Fig. 4.23. The PID requires only carrier gas. Routine maintenance includes lamp window cleaning, lamp replacement, lamp window seal replacement, and positioning. With a detection limit in the picogram range coupled to a linear dynamic range of 10⁷, the PID complements other detectors. The PID has also been configured in series before an FID to provide selective aliphatic hydrocarbon as well as aromatic hydrocarbon detection.

It is instructive to view the schematic of an EICD shown in Fig. 4.24 from the perspective of the analyte of interest as it emerges from the outlet of the PID and enters the reaction tube. The analyte is pyrolyzed in the reactor. In the sulfur mode, the analyte is oxidized using oxygen reaction

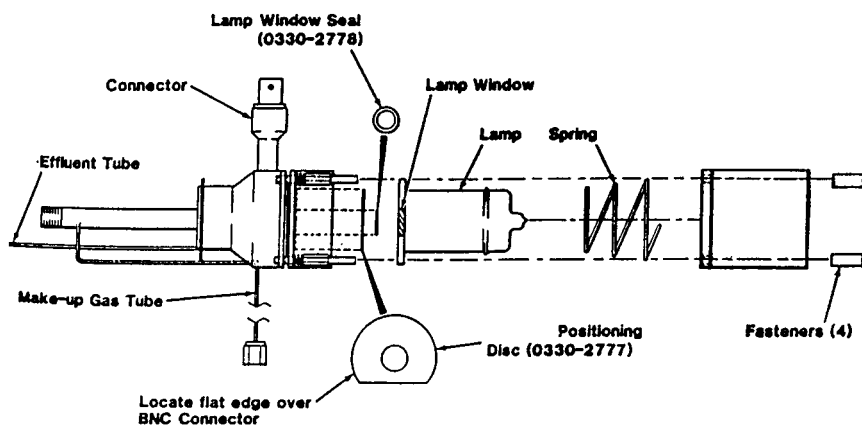


FIGURE 4.23 Schematic of a photoionization GC detector.

Understanding the basic parts and operation of an ELCD enhances an analyst's ability to properly use the detector.

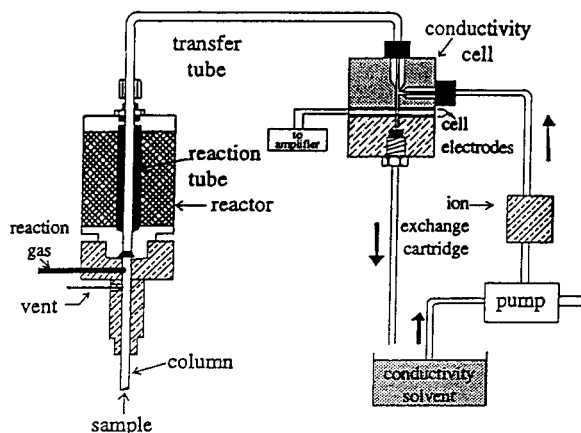


FIGURE 4.24 Schematic of the electrolytic conductivity GC detector.

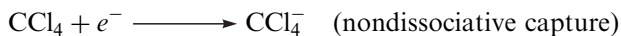
gas to sulfur dioxide. In the halogen and nitrogen modes, the analyte is reduced using hydrogen as a reaction gas to form HCl, HBr, HF, or NH_3 . The reaction tube acts as a catalyst to hasten reaction. The chemically converted analyte(s) of interest are swept through a transfer line into a conductivity cell. In the cell, the ionized analyte is dissolved in a flowing solvent and the change in conductivity is measured. To obtain a good response from the conductivity cell, the solvent flow and pH must be optimized. The sensitivity of the ELCD is inversely related to solvent flow rate. The pH of the solvent is controlled by passing it through an ion-exchange resin located in the solvent reservoir. The proper resin mixture will provide the correct pH for the solvent. The halogen and sulfur modes are acidic and require an acidic solvent; the nitrogen mode is basic and requires a basic solvent. Let us consider what happens to CIVOCs as they enter the ELCD. The reactor is made of nickel and is maintained at a temperature of $850\text{--}1000^\circ\text{C}$. The CIVOCs are reduced to haloacids by mixing them with hydrogen reaction gas. Nonhalogenated VOCs are reduced to methane, which is nonionic. The haloacids are dissolved in *n*-propanol and the change in solvent conductivity is measured in the cell. The ELCD is a bit more difficult to operate and to maintain in comparison to other GC detectors. The ELCD in the halogen mode has one chief competitor, the ECD.

38. WHAT IS AN ECD, HOW DOES IT WORK, AND WHY IS IT SO IMPORTANT TO TEQA?

The ECD introduced by Lovelock and Lipsky (64) and reviewed in two parts by Lovelock and Watson (65,66) is aptly described by Ewing (67) in the following manner:

For many years GC made use of TCDs almost exclusively, but with the advent of capillary columns, which are limited to smaller samples, greater sensitivity was required. One method of detection that can give greater sensitivity is a modification of the ionization chamber long used for radiation detection. The effluent from the chromatographic column is allowed to flow through such a chamber, where it is subjected to a constant flux of beta-ray electrons from a permanently installed radioisotope.

The inner wall of a cylindrically shaped cavity is lined with ^{63}Ni foil and a voltage is imposed between a pair of electrodes. This generates a standing current, I_b . When nitrogen or 95% Ar /5% methane gas is passed through, I_b is approximately 1×10^{-8} A. The ^{63}Ni is the source of beta radiation and thermal electrons are produced that do not recombine with either positive ions or neutral carrier gas molecules. Molecules of the analyte of interest that elute from the chromatographic column and contain electro-negative heteroatoms, such as chlorine, capture electrons and become negatively charged. These negatively charged gas-phase ions quickly combine with any positive ions present. A decrease in I_b results. As molecules of the electron-capturing analyte elute and are swept through the ECD, a negative peak results. The peak is inverted by the signal processing electronics to yield a positive peak like that obtained from all other GC detectors. The ECD requires about $30 \text{ cm}^3/\text{min}$ of gas flow. Because a typical WCOT flow rate is more than 10 times lower, makeup gas is required. More contemporary GCs provide the necessary makeup gas. This author has used the inlet from a second GC injection port as a source of makeup gas when makeup is not "plumbed in." The advantage of ^{63}Ni over ^3H (tritium was an earlier choice of beta radiation) lies in the much elevated temperature enjoyed by this isotope. A ^{63}Ni ECD can be safely taken to $T_{\text{ECD}} = 400^\circ\text{C}$. The following reactions help to explain the electron-capturing phenomena:



Many of the operational problems of ECD instability that plagued these detectors for so long have been overcome with advances in design. Contemporary GCs that have ECDs enable either N_2 or $Ar-CH_4$ to be used as carrier and/or makeup gas. The low-bleed WCOTs available today are a major contributor to the good stability of ECDs. Contamination even in this era of low-bleed WCOTs and well-designed ECDs is still a problem in the day-to-day laboratory operation. It is a wrong assumption to believe that all GCs that have ECDs are ready to use all of the time to meet the objectives of TEQA. This statement is consistent with this author's experience! Onuska and Karasek have identified the sources of ECD contamination that take the form of deposition of liquid phase and of dirt on the ECD electrodes (68). A contaminated ECD leads to loss of sensitivity, trailing peaks, and/or erratic baselines. The major sources of ECD contamination include the following:

1. Column bleed
2. Contaminated sample inlet including a dirty glass insert
3. Oxygen in carrier gas or a dirty carrier gas
4. Lack of a tight WCOT connection, thus allowing oxygen to enter

Figure 4.25 is a schematic for an ECD. EPA methods in general tend to require this detector for the determination of semivolatile to nonvolatile organochlorine analytes such as OCs and PCBs, whereby solvent extracts are introduced into the C-GC-ECD and water has, therefore, been completely eliminated.

39. HOW RESPONSIVE ARE ECDS?

Relative response factors (RRFs) for various organic compounds of interest to TEQA reveal an answer to this question. Listed in Table 4.11 are RRFs for most of the functional groups that one might encounter (68). The factor of 100 difference in RRFs between a trichloro organic and a tetrachloro organic is vividly demonstrated by referring to Fig. 4.26. This is a GC chromatogram obtained on an Autosystem Gas Chromatograph (Perkin-Elmer) that is interfaced to a HS-40 (Perkin-Elmer) automated headspace analyzer. The response of carbon tetrachloride (CCl_4) is significantly higher than either chloroform ($CHCl_3$) or trichloroethylene (TCE)! We close out our discussion of the ECD by showing two GC chromatograms from a C-GC-ECD in Fig. 4.27. These chromatograms show a method blank extract and a sample extract from the Soxhlet extraction of a fiberglass insulation sample brought to this author's laboratory for a quantitative determination of Aroclor 1260. The envelope of peaks show the presence of this particular

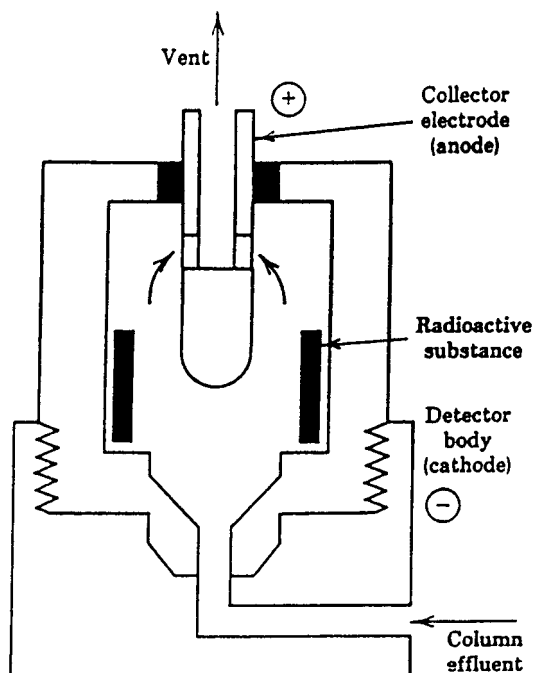


FIGURE 4.25 Schematic of the electron-capture GC detector.

commercial source of polychlorinated biphenyls (PCBs) in the insulation. This clear and almost interference-free chromatogram was obtained with no additional sample cleanup and shows the real value of a element specific GC detector. Imagine what this chromatogram would look like if it were injected into a C-GC-FID?

TABLE 4.11 Relative Response Factors and Nature of the Electron-Capturing Functional Group

RRF	Electron-capturing functional group
10^0	Aliphatic hydrocarbons, aromatic hydrocarbons
10^1	Esters, ethers
10^2	Monochloro, monofluoro, aliphatic alcohols, ketones
10^3	Dichloro, difluoro, monobromo derivatives
10^4	Trichloro, anhydrides
10^5	Monoiodo, dibromo, nitro derivatives
10^6	Diiodo, tribromo, polychlorinated aromatics

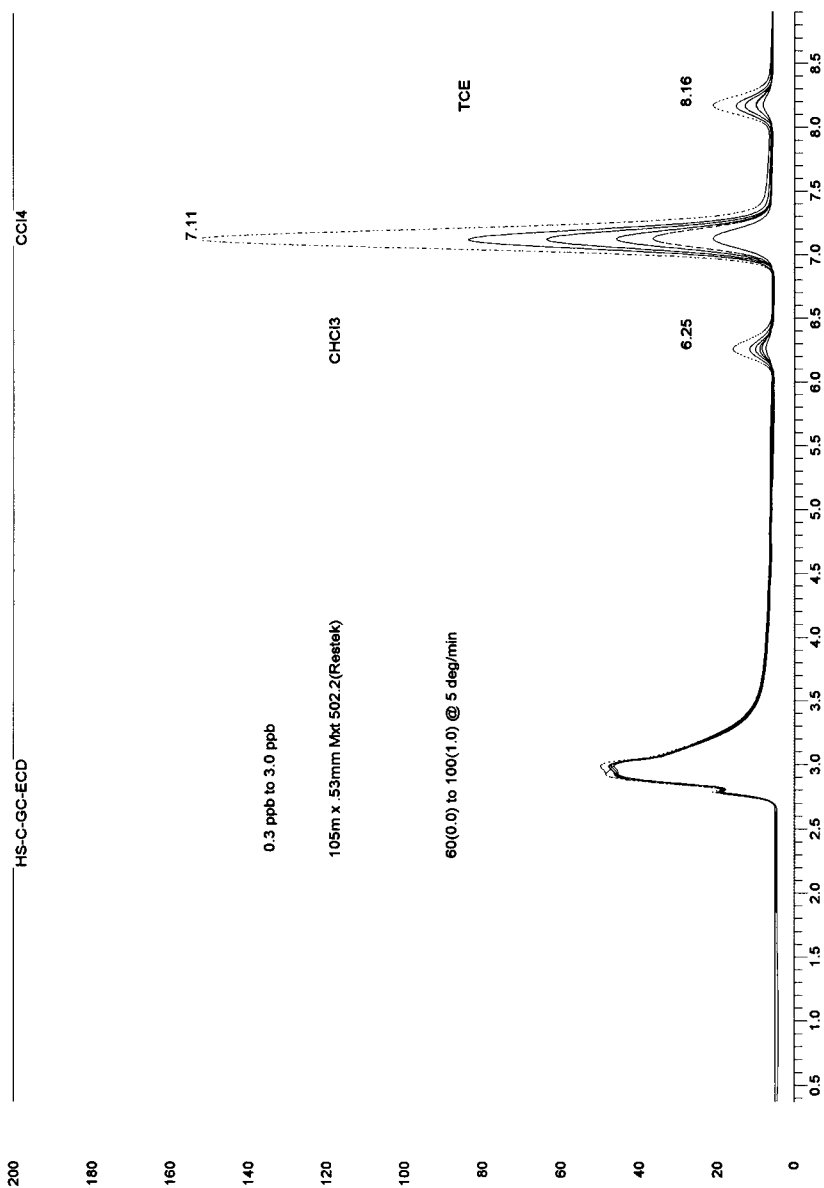


FIGURE 4.26 Capillary GC-ECD chromatogram for the separation and detection of chloroform, carbon tetrachloride, and trichloroethylene.

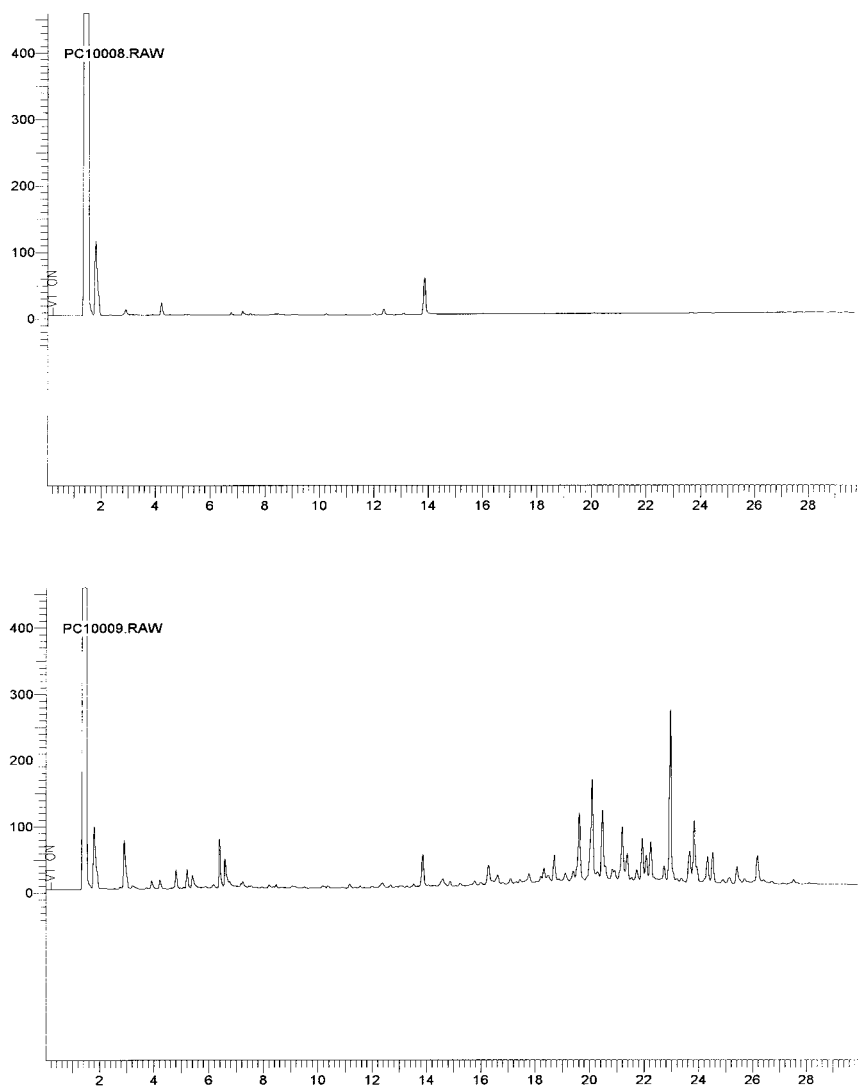


FIGURE 4.27 Comparison of capillary GC-ECD chromatograms between a method blank and building fiberglass insulation suspected of containing AR 1260.

40. ARE THERE OTHER ELEMENT-SPECIFIC DETECTORS?

Yes indeed, and we make brief mention of these. We must ask, however, the following question. What other chemical elements are incorporated into organic molecules that are of interest to TEQA? Priority pollutant organic compounds of environmental interest that incorporate the elements nitrogen, phosphorus, and sulfur answer this question. GC detectors developed for these elements are of a more recent vintage and include thermionic, flame photometric, and chemiluminescence. In addition, the atomic emission spectroscopic detector (AES) has been recently developed commercially for organometallics. The AES can be tuned to a specific emission wavelength for a particular metal. Development of C-GC-AES techniques have advanced the analytical chemistry of trace metal speciation.

The thermionic specific detector (TSD), also called the alkali flame ionization detector, is really a FID with a bead of an alkali metal salt such as Rb or Cs. A schematic is shown in Fig. 4.28. The TSD shows

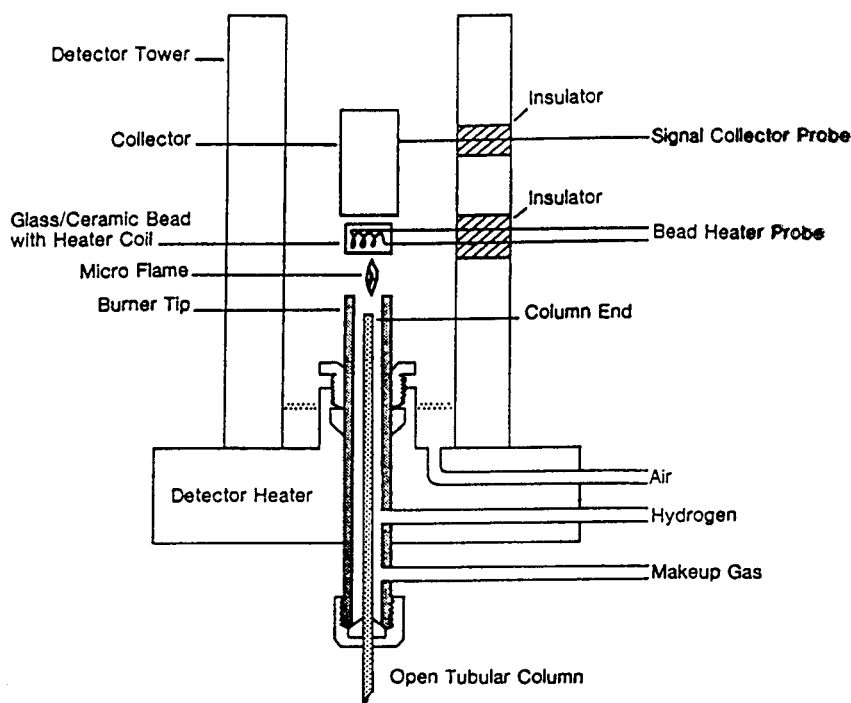


FIGURE 4.28 Schematic of the thermionic GC detector.

enhanced sensitivity for organic compounds that contain the elements nitrogen and phosphorus. This author has used a GC that incorporated a TSD to measure the extent of contamination of groundwater in an aquifer somewhere in Maine with *N,N*-dimethylformamide (DMF). Figure 4.28 shows that the bead can be heated to a red glow and the heating of the bead has led to increased TSD stability. A negative polarizing voltage is applied to the collector. The ion current generated by the thermionic emission can be related to the mass of, for example, DMF eluted from the WCOT.

The flame photometric detector (FPD) operates on the principle that phosphorus- and sulfur-containing organics compounds eluted from the GC column emit characteristic green and blue colorations, respectively, in hydrogen-rich, H_2 -air flames. A schematic of a dual FPD is shown in Fig. 4.29. This design first developed at Varian Associates burns the organic solute during passage through the lower flame and then excites the HPO species and causes it to emit at 526 nm for phosphorus-containing organic compounds or S_2 at 394 nm for sulfur-containing organic compounds in the higher flame (69). These species emit characteristic wavelengths through an optical window through various cutoff filters to a photomultiplier tube as shown in Fig. 4.29. Hydrogen gas enters from the side and mixes with air and carrier gas to sustain a hydrogen flame. The design criteria in the dual-flame FPD is designed, for example, to maximize the flame photometric emission of HPO while minimizing hydrocarbon emissions and interferences due to S_2 .

This author was once fortunate to have access to a gas chromatograph (Model 3400, Varian) that incorporated both a TSD and a FPD. This instrumental configuration enabled this author to conduct a comprehension study of the isolation and recovery of representative OPs via reversed-phase solid-phase extraction (SPE) (70). Figure 4.30 is a schematic of the single-injector/dual-column/dual-detector GC configuration used to conduct the study. In order to provide the necessary makeup carrier gas to both detectors, stainless-steel tubing from the inlet of the first injector was connected to the detector inlets as shown in Fig. 4.30. A dual-GC chromatogram, shown in Fig. 4.31, was obtained using this dual GC just described. A DB-5 and a DB-608 WCOT column were used to separate a mixture that contains seven OPs and the internal standard, triphenyl phosphate. Note that the elution order changes somewhat between both columns. The instrumentation used and the instrumental conditions employed in this study are given in Table 4.12. The specific operational parameters shown in this table reveal much about how to operate both the TSD and FPD GC detectors. The instrumentation was of a late 1980s vintage and reveals the aspects of GC instrumentation that have changed during the past 15 years. The fact

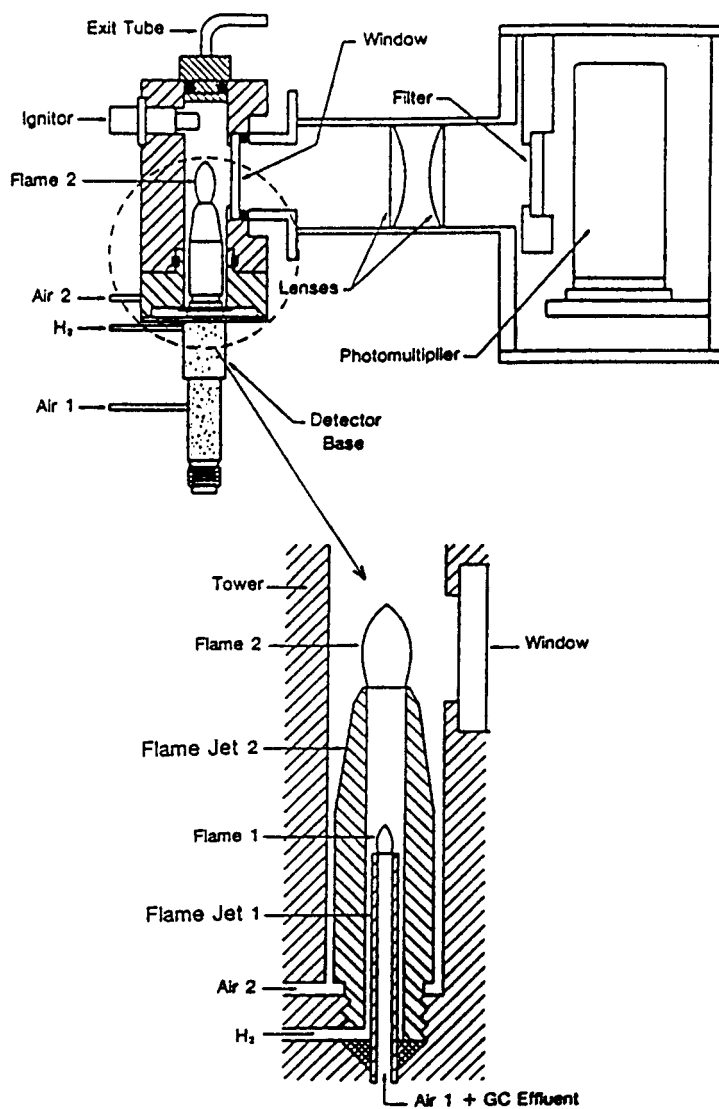


FIGURE 4.29 Schematic of the flame photometric GC detector.

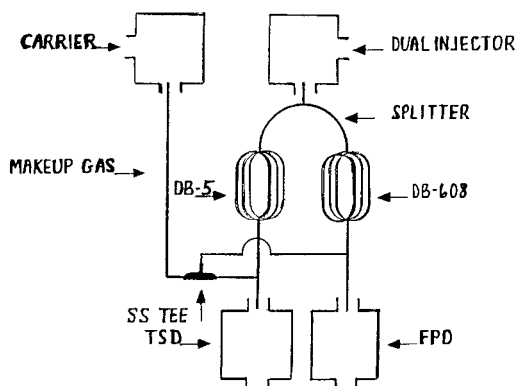


FIGURE 4.30 Dual mega-bore capillary column configuration used to study the isolation and recovery of organophosphorus pesticides.

that these OPs could be isolated, recovered, and detected at trace concentration levels is most significant and cannot change!

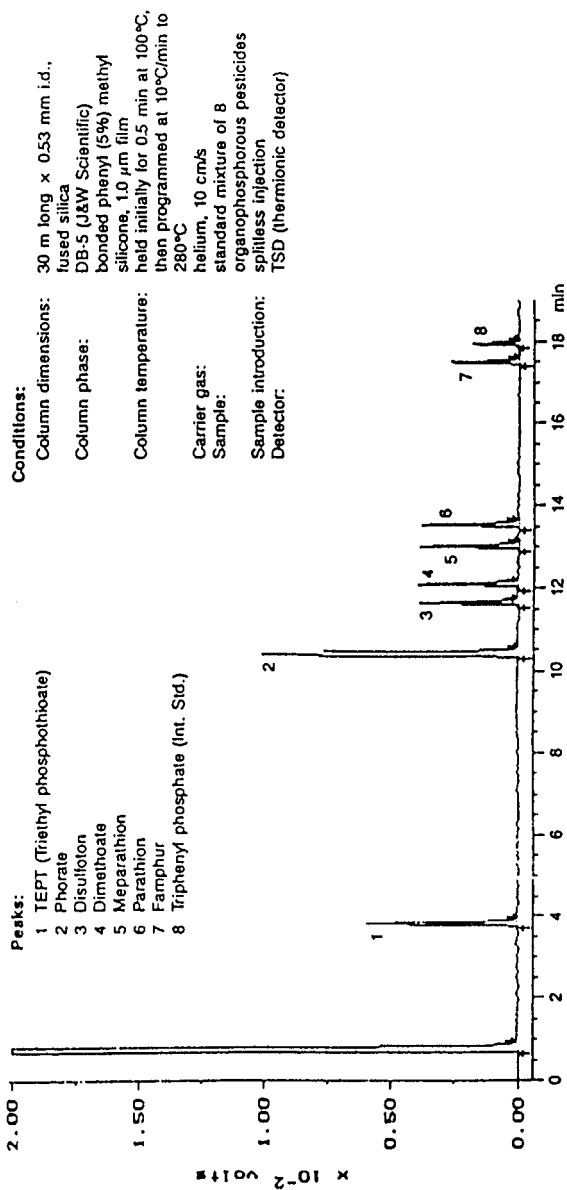
41. WHAT ABOUT ANY RECENTLY DEVELOPED ELEMENT-SPECIFIC GC DETECTORS?

Two such detectors will be discussed here, the pulsed-discharge detector (PDD) manufactured by VICI (Valco Instruments Company, Inc.) and the chemiluminescence detector, manufactured by Sievers Instruments. Both detectors are of a more recent vintage. The PDD has the potential of replacing either the ECD or FID, and also the PID, depending on the application to TEQA. The PDD is a nonradioactive pulsed-discharge ionization detector and was designed for ease of installation to the Model 6890 GC (Hewlett-Packard, now Agilent Technologies). A stable, low-power, pulsed DC discharge in helium is the source of ionization. Solutes eluting from the cap column flowing in the opposite direction to that of the flow of helium from the discharge zone are ionized by photons from the helium discharge above. Electrons from this discharge are focused toward the collector electrode by the two bias electrodes. A schematic of the PDD is shown in Fig. 4.32. Photoionization by radiation is the principal mode of ionization and arises from the transition of diatomic helium to the dissociated monatomic He ground state. In the electron-capture mode, the PDD is a selective detector for monitoring high-electron-affinity compounds such as CIVOCs, OCs, PCBs, and so forth. GCs that incorporate the PDD would be capable of IDLs down to low-ppb concentration levels. In this mode, helium and

TABLE 4.12 GC Operation Conditions Used to Conduct Dual Mega-Bore Capillary GC-NP Detection

Gas chromatograph	Varian 3400 with Varian 8034 autosampler
Mega-bore splitter	Supelco direct injector tee, silanized with appropriate fittings
Data station	Varian 604 with Varian IIM interface using the dual-column software option
Printer	Hewlett-Packard Think Jet printer
WCOT	30 m × 0.53 mm DB-5 and 30 m × 0.53 mm DB-608 from J & W Scientific
TSD and flow characteristics	300°C, air at 175 cm ³ /min, H ₂ at 4.5 cm ³ /min, carrier (N ₂) at 5 cm ³ /min, makeup (N ₂) at 25 cm ³ /min
FPD and flow characteristics	300°C, air 1 at 80 cm ³ /min, air 2 at 170 cm ³ /min, H ₂ at 140 cm ³ /min, carrier (N ₂) at 5 cm ³ /min, makeup (N ₂) at 25 cm ³ /min.

Organophosphorous Pesticides Dual Wide-Bore Capillary/TSD



Organophosphorous Pesticides
Dual Wide-Bore Capillary/FPD

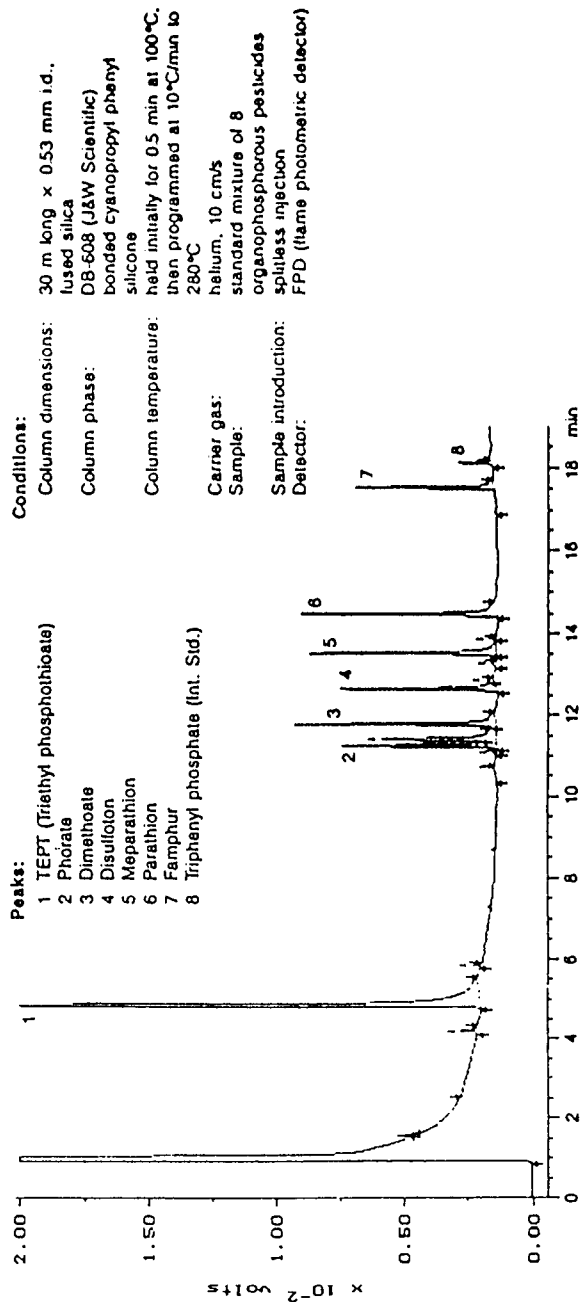


FIGURE 4.31 Dual chromatograms from a single injection of a reference standard containing organophosphorus pesticides.

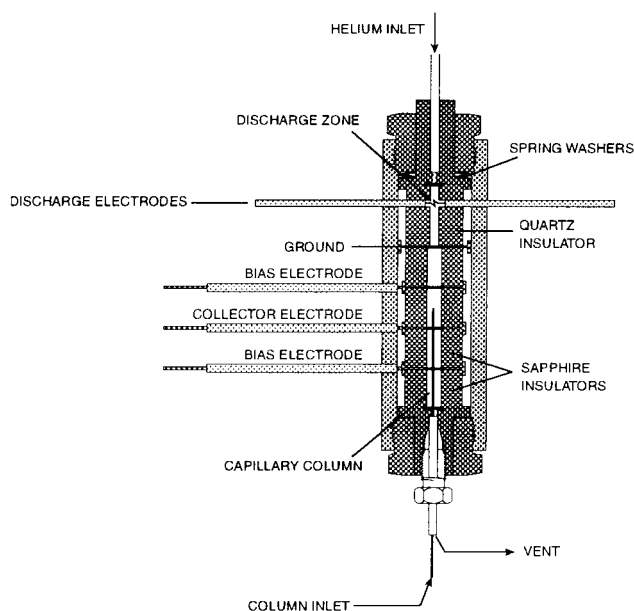


FIGURE 4.32 Schematic of the pulsed-discharge GC detector.

methane are introduced just upstream from the column exit. In the helium photoionization mode, the PDD is a universal, nondestructive, highly sensitive detector. In this mode, the PDD is a suitable replacement for a conventional FID. When a dopant is added to the discharge gas, the PDD also functions as a selective photoionization detector. Suitable dopants include Ar for organic compounds, Kr for unsaturated compounds, or Xe for polynuclear aromatics. A drawing of the PDD as it might appear when being installed into a 6890 GC is shown in Fig. 4.33. This is a good example of how existing gas chromatographs can be “retrofitted” with more newly developed GC detectors as the need arises.

Because ECDs require radioactive sources, the pulsed-discharge electron-capture detector, PDECD, is a relatively recent alternative. A cross section of a PDECD that incorporates methane as a dopant is shown in Fig. 4.34. A detailed description of the design of this detector is available (71). Cai et al. have described their recent developments while studying the influence of discharge current, bias and collecting voltages, and the chemical nature of the dopant gas while comparing conventional ECDs to the PDECD (71). The pulsed discharge in pure helium produces high-energy photons according to

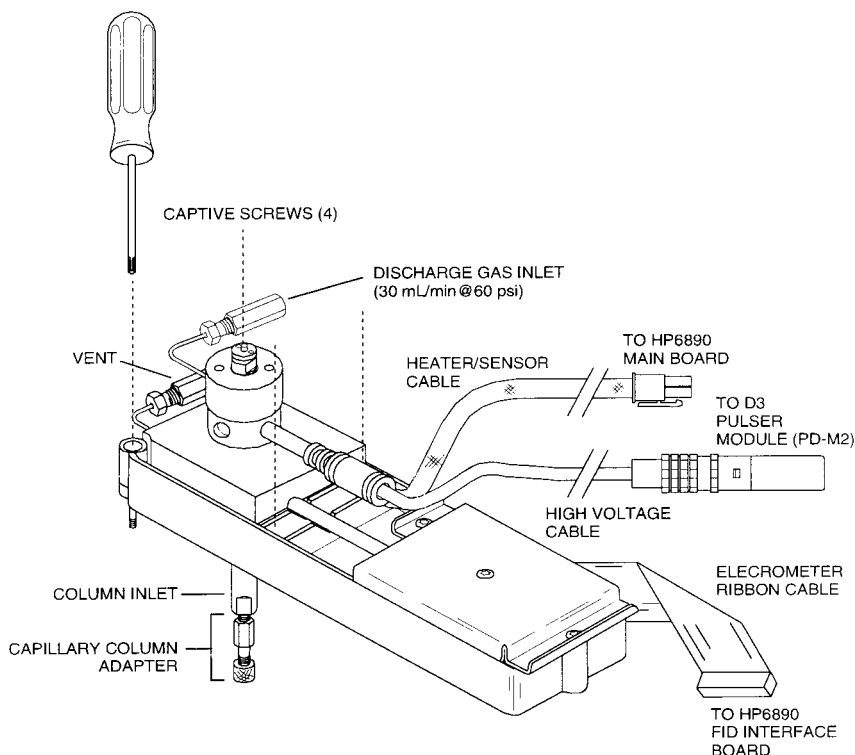
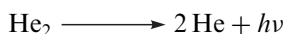


FIGURE 4.33 Drawing to show how the pulsed-discharge GC detector is installed onto a Model 6890 (Hewlett-Packard) gas chromatograph.



If methane is added downstream from the discharge, free electrons are produced. The dopant also thermalizes the electrons, making them more “capturable” by analytes. Analytes capture these thermalized electrons and reduce the standing current I_b according to

$$\frac{I_b - I_e}{I_e} = K_{\text{EC}}[\text{AB}]$$

where I_b is the standing current without analyte, I_e is the current with the electron-capturing analyte AB present, K_{EC} is a capture coefficient for AB, and $[\text{AB}]$ represents the concentration of AB. This equation was first developed by Wentworth et al. for an ECD model (72). Thermal electrons are

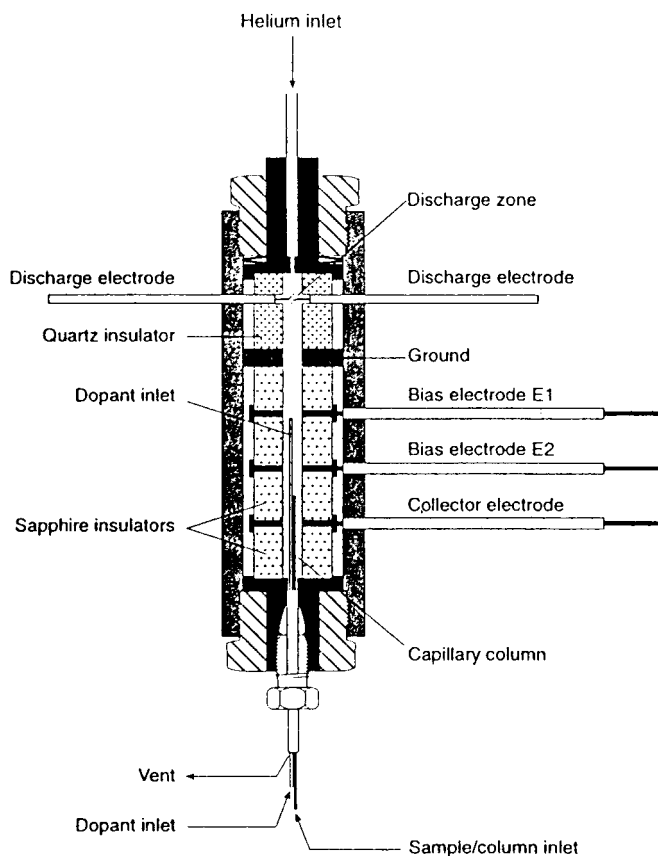


FIGURE 4.34 Cross section of the pulsed-discharge electron-capture GC detector.

first formed in the discharge zone as methane undergoes photoionization according to



These primary electrons from photoionization become thermalized:



some electrons and positive ion species, P^+ , recombine to form neutral species according to



The dissociative and nondissociative mechanisms discussed earlier for the ECD also apply to the PDECD. The only difference in kinetic models is that of the first step of electron formation. The capture coefficient K_{EC} can then be defined in terms of the various rate constants for these various reactions. The collection of thermal electrons constitutes I_b for the PDECD. This standing current decreases during passage of an electron-capturing analyte through the detector and is responsible for the PDECD signal (73). The use of a pulsed discharge rather than a continuous discharge serves to impart a higher energy to the cell because energy is dissipated during a very short period. The density of ions should be kept low for the charged species to be sustained in the reaction region. Pulsing also keeps the average potential to near zero, thus allowing what Wentworth et al. call a "field-free" condition to be realized shortly after the discharge takes place (73).

A comparison of the relative response factors (RRFs) for a selective number of CIVOCs that have been discussed earlier is given in Table 4.13 (71). The PDECD is as responsive if not more responsive than the ^{63}Ni -ECD for aliphatic CIVOCs; however, the PDECD becomes somewhat less responsive for mono- and dichlorobenzenes. A comparison was also made between a conventional ^{63}Ni -ECD and the PDECD with respect to satisfying the criteria posed in EPA Method 608 for OC pesticides. GC chromatograms for the injection of a multicomponent OC standard including the TCMX and DCBP surrogates discussed earlier are shown for a standard, sample and matrix spike for both detectors in Fig. 4.35. Note that the authors plot the ratio $(I_b - I_e)/I_e$ versus chromatographic run time in the figure (71). The difference in WCOT column polarity is responsible for the differences in elution order. It would appear that the PDECD is a viable

TABLE 4.13 Comparison of RRFs for PDECD and ^{63}Ni -ECD

CIVOC	RRF (PDECD)	RRF (^{63}Ni -ECD)
CCl_4	10,000	10,000
PCE	5,000	4,000
CHCl_3	660	470
TCE	570	380
Chlorobenzene	13	45
1,3-Dichlorobenzene	13	24
1,2-Dichloroethane	1.2	0.8
CH_2Cl_2	0.3	0.3

Note: PCE = tetrachloroethene; TCE = trichloroethene.

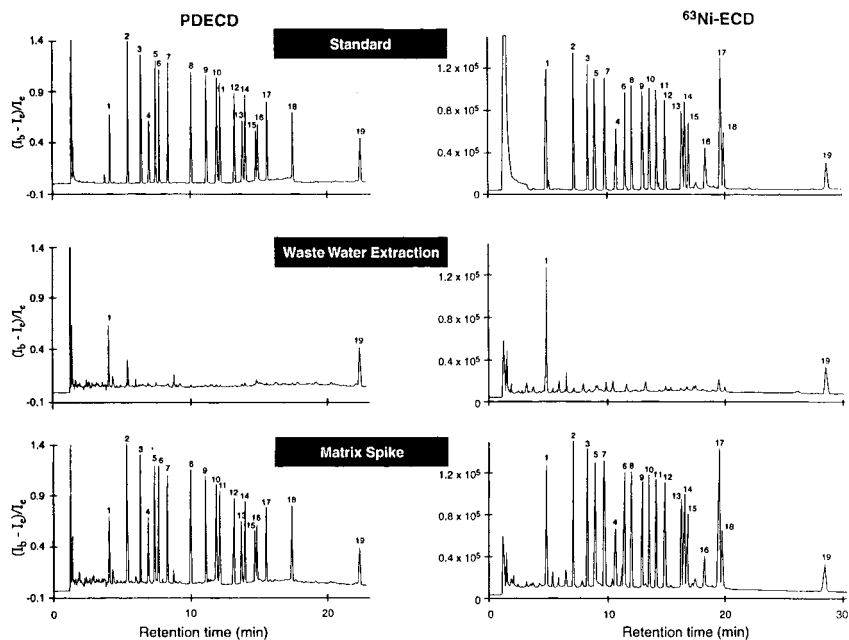


FIGURE 4.35 Chromatograms that compare the response of the PDECD to a conventional ^{63}Ni -ECD under the same conditions.

alternative to the ECD. One other element-specific GC detector needs to be discussed that involves chemiluminescence.

42. OK, BUT WHAT ABOUT CHEMILUMINESCENCE?

Chemiluminescence can be defined as the spontaneous emission of light by chemical reaction. In the sulfur chemiluminescence detector (SCD), organo-sulfur compounds that elute from a WCOT are combusted in a hydrogen-rich flame to produce sulfur monoxide (SO) among other products. This is the same process that occurs in a FID. These combustion products are collected and removed from the flame using a ceramic sampling tube (probe) interface and transferred under a vacuum through a flexible tube to the reaction chamber of the SCD. Sulfur monoxide is detected by an ozone/SO chemiluminescent reaction to form electronically excited sulfur dioxide (SO_2^*), which relaxes with emission of light in the blue and the ultraviolet regions of the electromagnetic spectrum according to (74)



One model of a SCD is manufactured by Sievers Instruments, Inc. and readily adapts to existing GCs. A more recent development from Sievers is the nitrogen chemiluminescence detector (NCD). Organonitrogen compounds that elute from a WCOT enter a ceramic combustion tube in a stainless steel burner. The hydrogen and oxygen plasma in the combustion tubes convert all organonitrogen compounds to nitric oxide at temperatures greater than 1800°C according to



Nitric oxide reacts with ozone to form electronically excited nitrogen dioxide according to



Excited NO_2 emits light in the red and infrared regions of the electromagnetic spectrum, from 600 to 3200 nm when it relaxes to its ground state. The light emitted is directly proportional to the amount of nitrogen in the sample (75). Figure 4.36 depicts a schematic diagram for either a SCD or a NCD connected to a GC. Note that a burner head is attached to the existing detector port much like a FID. The combustion products are directed to

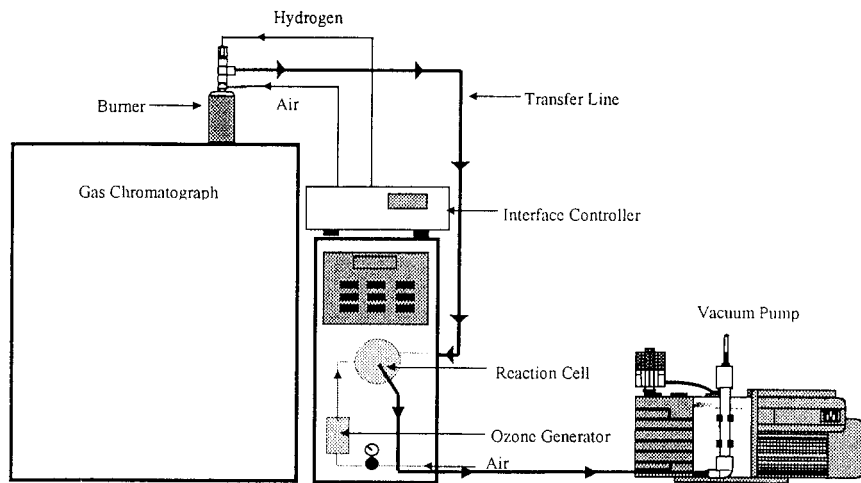


FIGURE 4.36 Schematic diagram of a sulfur or nitrogen chemiluminescence GC detector.

a reaction cell where ozone can be generated and added. A photomultiplier tube, not shown in the schematic, is incorporated into the detector. Comparisons have been made between the NCD and the TSD. Element specific GC detectors are in general very sensitive and are quite useful for target specific determinations; however, the most widely used GC detector and the one that satisfies the most EPA methods is the mass spectrometer (MS) and the success that has been achieved in interfacing a GC with a “mass spec.” This interface was the first successful hyphenated method, a term coined by Hirshfeld to describe how two established instruments could be “married” such that the whole is greater than the sum of the parts.

43. OF THE PLETHORA OF MASS SPECS, WHICH IS MOST USEFUL TO TEQA?

Because volumes have been written concerning mass spectrometry over the past 40 years, our approach here is to focus on the type of “mass spec” instrumentation required to perform EPA methods and those systems can be described as being of a low-resolution nature and the most affordable. The quadrupole mass filter or mass selective detector (MSD) and quadrupole ion trap mass spectrometer (ITD) fit this criteria and will be the only mass specs discussed. Onuska and Karasek have given a good definition and description of the importance of gas chromatography-mass spectrometry (GC-MS) to TEQA (76):

In its simplest form the mass spectrometer performs three basic functions as a GC detector. These functions are to deliver a sample into the ion source at a pressure of 1×10^{-5} torr to produce ions from neutrals molecules, and to separate and record a spectrum of ions according to their mass-to-charge ratios (m/z) and relative abundance. If a WCOT column represents the heart of the system, the mass spectrometric detector is the brain at the highest intelligence level. It is capable of providing both qualitative and quantitative data by means of spectral interpretation procedures developed to identify and quantify individual components in a mixture or of measuring a specific compounds or group of positional isomers by means of selective ion monitoring (SIM).

For a more complete description, as well as ease of comprehension of the broad field of mass spectrometry including mass spectral interpretation, please refer to Ref. (77). A somewhat oversimplified schematic of a quadrupole MSD is shown in Fig. 4.37 and depicts the trajectory of a resonant ion that makes it through the four rods of the quadrupole and a nonresonant ion that does not. The effluent from a WCOT can be fitted in

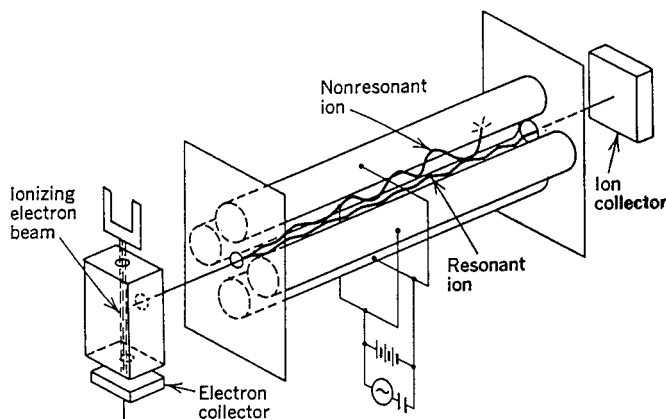


FIGURE 4.37 Schematic diagram of a quadrupole mass selective filter as found in typical environmental testing labs.

such a way that the column outlet is positioned just in front of the electron beam. This beam is produced by “boiling off” electrons from a heated filament and accelerating the electrons to the collector. Molecular and fragment ions that are produced from this “electron impact” are accelerated through a series of electric fields and enter the center of the four rods. Diagonally opposite rods are connected together electrically and to radio-frequency (RF) and DC voltage sources as indicated in Fig. 4.38. The applied voltages on one set of quadrupole rods is 180° out of phase with the applied voltages on the other set of rods. The quadrupole MSD is scanned by increasing the magnitude of the RF amplitude and DC voltages while maintaining a fixed ratio of the two. It is this ratio of the RF to the DC voltage that determines the resolving power of the quadrupole MSD. Sweeping the voltages from a preestablished minimum to a maximum value, while keeping the ratio of RF to DC voltage constant, will provide a mass spectrum of resolution $R = 1$ for the molecular ion, if any, and any and all mass fragment ions from electron impact of the chromatographically resolved component in a mixture. This scan occurs very fast and enables a large number of scans to be recorded as the chromatographically resolved component enters the MSD. A plot of ion abundance versus m/z gives a mass spectrum and serves to confirm the presence of a particular analyte. C-GC-MS therefore serves to confirm the identity of an analyte and thus complements C-GC with element-specific detection. This is one of the most powerful trace analytical instrumentation concepts devised in the twentieth century!

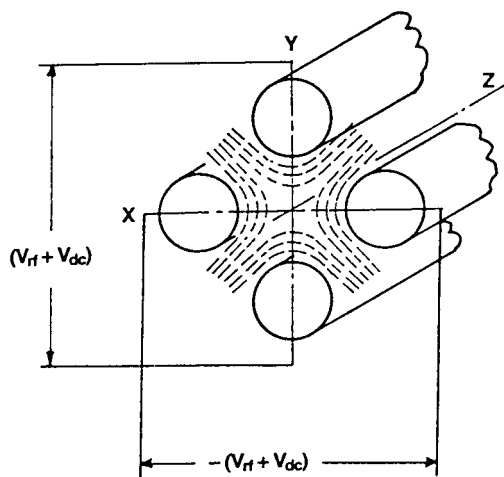


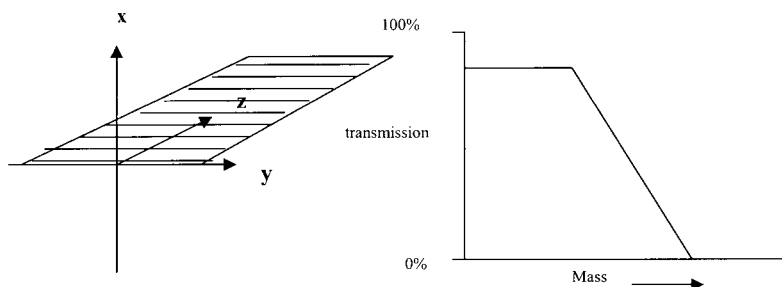
FIGURE 4.38 Schematic showing the radio-frequency and direct-current voltages applied to the diagonally opposite rods.

Miller and Denton have likened the quadrupole MSD filter to a “tunable variable bandpass mass filter” and contrast this with “true mass spectrometers” that resolve ions by dispersing them in either space, such as a magnetic sector instrument, or in time such as that of a time-of-flight instrument (78). These authors suggest that the combination of a time-independent DC and a time-dependent RF potential applied to the four rods in essence act like a mass band filter. With reference to Fig. 4.39 and with the center of the quadrupoles at the origin of a set of Cartesian coordinates, the rods act as a high-pass mass filter for ions in the x - z plane while acting as a low-pass mass filter in the y - z plane. An ion must remain stable in both the x - z and y - z planes to make it from the ion source to the detector. It must be light enough so as not to be eliminated by the low-pass filter operating in the y - z plane yet not so light as to be eliminated by the high-pass mass filter that operates in the x - z plane. This condition of mutual stability allows a narrow mass range to “make it through.”

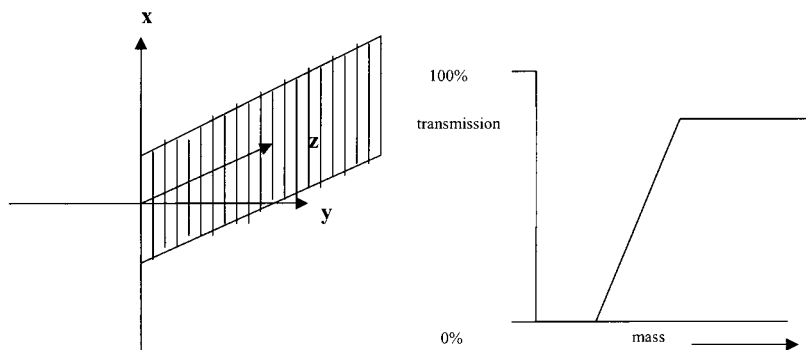
44. CAN WE PREDICT WHAT m/z VALUES ARE STABLE THROUGH THE QUADRUPOLE RODS?

Yes we can! We must, however, delve into the physics of the quadrupole. For the classical hyperbolic mass filter, the potential Φ for any time t can be described by

The quadrupole acts as a low-pass mass filter in the y-z plane



The quadrupole acts as a high-pass mass filter in the x-z plane



The quadrupole as a bandpass mass filter results from overlap of the high-pass mass filter of the x-z plane and the low-pass mass filter of the y-z plane

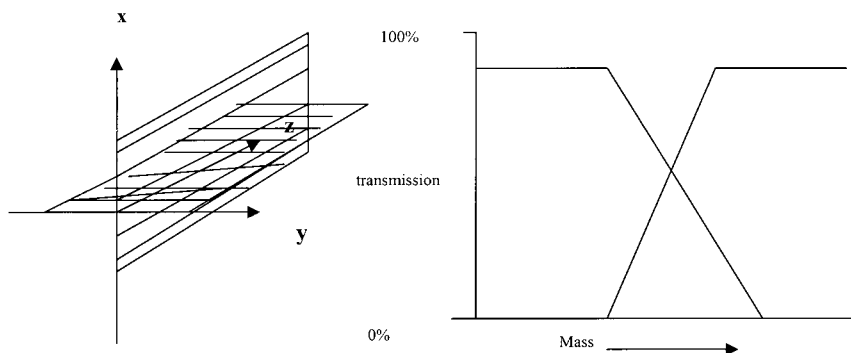


FIGURE 4.39 Conceptual framework to help explain the mass filtering properties of the quadrupole mass spectrometer. (From Ref. 78.)

$$\Phi = [V_{\text{DC}} + V_{\text{RF}} \cos(\omega t)] \frac{x^2 - y^2}{2r_0^2}$$

where V_{DC} is the time-independent DC potential, V_{RF} is the magnitude of the applied RF amplitude, ω is the angular frequency where $\omega = 2\pi f$ with f being the RF frequency, x and y are Cartesian coordinates defined with respect to Fig. 4.39, and r_0 is the distance above the z axis. The RF frequency is typically around 1.5 MHz. The partial derivative of this potential gives the magnitude of the electric field in all three directions, x , y , and z . The electric field in the x direction is obtained by taking the partial derivative with respect to x of the potential Φ as is shown by

$$E_x = -\frac{\partial \Phi}{\partial x} = -[V_{\text{DC}} + V_{\text{RF}} \cos(\omega t)] \frac{x}{r_0^2}$$

The electric field in the y direction is obtained similarly as follows:

$$E_y = -\frac{\partial \Phi}{\partial y} = [V_{\text{DC}} + V_{\text{RF}} \cos(\omega t)] \frac{y}{r_0^2}$$

The electric field in the z direction is obtained in a similar manner as well; however, because there is no dependence of the potential in this direction, we get

$$E_z = -\frac{\partial \Phi}{\partial z} = 0$$

The force, F_x , exerted on a charged particle in the x direction by the magnitude of the electric field is equal to the product of the electric field E and the charge e such that

$$F_x = -[V_{\text{DC}} + V_{\text{RF}} \cos(\omega t)] \frac{ex}{r_0^2}$$

The force F_y exerted in the y direction is likewise found:

$$F_y = [V_{\text{DC}} + V_{\text{RF}} \cos(\omega t)] \frac{ey}{r_0^2}$$

There is no force exerted in the z direction, so

$$F_z = 0$$

The force exerted on the ion is also equal to the product of its mass and its acceleration, expressed in terms of the x coordinate, we obtain

$$F_x = ma = m \frac{d^2x}{dt^2}$$

Rearranging this equation, we have

$$m \frac{d^2x}{dt^2} - F_x = 0$$

Substituting for F_x , we then have

$$m \frac{d^2x}{dt^2} + [V_{DC} + V_{RF} \cos(\omega t)] \frac{ex}{r_0^2} = 0$$

We can develop similar relationships for the y direction, and upon substituting for F_y , we have

$$m \frac{d^2y}{dt^2} - [V_{DC} + V_{RF} \cos(\omega t)] \frac{ey}{r_0^2} = 0$$

By letting $\xi = \omega t/2$ and designating any x , y , or z coordinate as u , the derivation as presented by March and Hughes enables us to arrive at the canonical form of the Mathieu equation (79):

$$\frac{d^2u}{d\xi^2} + [a_u + 2q_u \cos(2\xi)]u = 0$$

where the terms a_u and q_u are defined as follows:

$$a_u = \frac{8V_{DC}}{\omega^2 r_0^2 (m/e)}$$

$$q_u = \frac{4V_{RF}}{\omega^2 r_0^2 (m/e)}$$

Solutions to the Mathieu equation completely describe the trajectory of an ion in terms of each ion's initial conditions. Without any force acting along the z axis, the position and velocity of an ion along its z axis is unaffected by any potential applied to the rods. The use of rods of a

hyperbolic cross section leads to equations of motion that contain no cross-coordinate terms (78).

Solutions to the Mathieu equation reveal regions of a - q space whereby the ion is bound and therefore the ion is able to remain stable within the region between the four rods and makes it way through to the conversion dynodes and electron multiplier detector. This is the so-called stability region. Note that parameters a and q are, in essence, reduced parameters and enable one to simplify the concepts. For a given mass to charge ratio m/e , a fixed distance r_0 , and for a fixed RF frequency ω , a and q depend only on V_{DC} and V_{RF} , respectively. In practice, quadrupoles are usually operated in a manner such that the values of parameters a and q are always related by a simple ratio. This condition is established by ensuring that the applied DC potential is always some fraction of the applied AC potential. The ratio is held constant, irrespective of the magnitude of V_{DC} or V_{RF} . Holding the ratio of V_{DC} to V_{RF} constant defines a set of points in a - q space called an operating line or mass scan line. If a is divided by q for a given m/e , this ratio equal to $2(V_{DC}/V_{RF})$ is obtained. A plot of a versus q is shown in Fig. 4.40 along with two mass scan lines that differ in their slopes. Resolution is increased by slightly changing the slope of the mass scan lines such that it is possible to achieve a desired resolution. Let us review what we mean by mass-spectrometric resolution so that we do not confuse this term with chromatographic resolution.

45. WHAT IS MASS-SPECTROMETRIC RESOLUTION ANYWAY?

Mass-spectrometric resolution is defined as the ratio of a given mass m to the smallest discernable difference in mass, Δm , that can be measured. Mathematically, we have

$$R = \frac{m}{\Delta m}$$

A numerical expression can be obtained from this ratio where m and Δm are m/e values of two adjacent peaks in the mass spectrum (80). Table 4.14 suggests, for a given mass and resolution, to what extent one can resolve close m/e values.

In practice, V_{DC} can be varied from -250 V to $+250$ V and V_{RF} can vary from -1500 V to $+1500$ V. The magnitude of V_{RF} is approximately six times that of V_{DC} . The mass scan line is swept across the bandpass region by changing V_{DC} and V_{RF} while keeping their ratio constant. A voltage increase is equivalent to sliding the mass scale shown in Fig. 4.40 upward

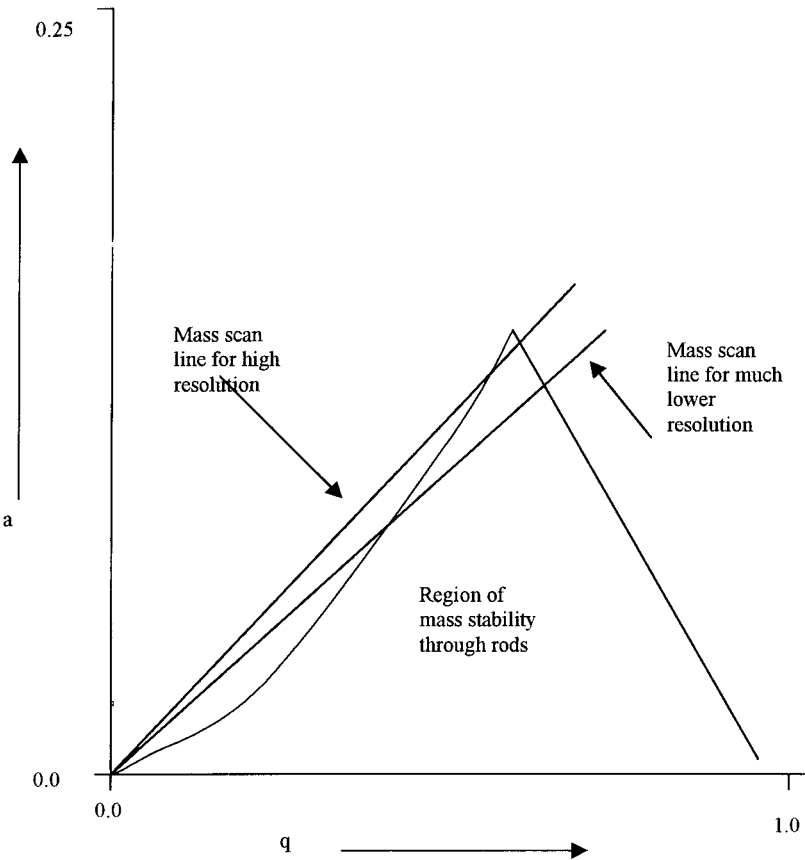


FIGURE 4.40 Stability diagram of a – q space showing regions where a stable ion trajectory exists.

TABLE 4.14 Relationship Between Mass-Spectrometric Resolution and Mass

m	R	$m - \Delta m$	m	$m + \Delta m$
10	10	9	10	11
100	10	90	100	110
10	100	9.9	10.0	10.1
100	100	90.9	100.0	100.1
10	1000	9.99	10.0	10.01
100	1000	90.99	100.00	100.01

and to the right along the mass scan line. Sweeping the voltage applied to the rods thus provides a convenient method of scanning the bandpass region of the MSD.

46. THE MATH IS OK, BUT WHAT REALLY HAPPENS TO M^+ WHEN IT IS PROPELLED INTO THE CENTER OF THE QUADRUPOLE?

The mathematics just discussed provides a theoretical basis to understand how the quadrupole MSD selects specific m/z values with the desired degree of mass-spectrometric resolution. Two books (81,82) provide sufficient detail for the reader who desires a more in-depth treatment on theory. To answer the question, let us consider “riding the back” of a molecule that has lost its valence electron due to electron impact. This molecule ion can be abbreviated M^+ , and by taking this viewpoint, it will enable us to look at how this species gets “knocked around” by varying the applied voltages to the rods. This pedagogical approach was first used by the Finnigan MAT Institute as part of their training program (83).

Figure 4.41 is a plot of the magnitude of the V_{DC} and V_{RF} voltages applied to the rods of a MSD. The dashed line shows how the combined DC and AC voltages vary on the first set of diagonally opposite rods, labeled 1 and 4, while the solid line represents the combined DC and AC voltages applied to the other set of rods, labeled 3 and 4. Our M^+ begins at time t_0 and we follow four subsequent time frames or “snapshots.” The applied voltages for each of six snapshots are given in Table 4.15 for this exercise. The snapshots are shown in Fig. 4.42. Three mass fragment ions of m/z 4, 100, and 500 are propelled into the center of the four rods. At time t_0 with rods 1 and 4 at 20 V and rods 2 and 3 at -20 V, all three fragment ions are attracted to rods 2 and 3 while being repelled by rods 1 and 2. At time t_1 , rods 1 and 4 have become even more positive while rods 2 and 3 have become even more negative. This change in the rod voltages accelerates all three ions to rod 2. The lightest ion, that with m/z 4 crashes into rod 2. At time t_3 , rod 2 is suddenly made quite positive and ions of m/z 100 and 500 are repelled as shown. At time t_5 , rod 2 is made quite negative and m/z -500 is too heavy to change direction away from the rod and crashes into it. The ion with m/z -100 is now heading toward rod 1 and is suddenly repelled by this rod whose voltage is +140 and is steered toward the center of the rods as shown. Figure 4.43 shows the somewhat helical path taken by the ion whose m/z is 100 through the quadrupole and to the detector. This ion would be considered bound and reside in $a-q$ space. Beyond this more or less oversimplified concept, in practice, the operation can make minor

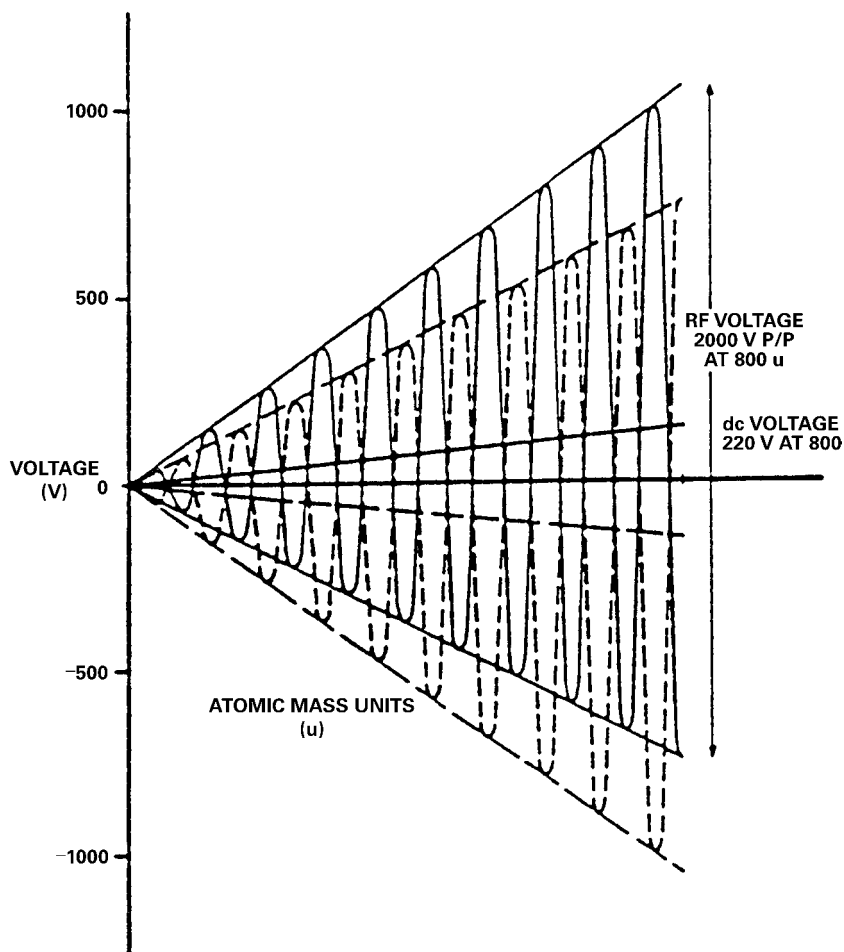


FIGURE 4.41 Plot of the magnitude of the V_{DC} and V_{RF} applied to the rods of a mass selective detector.

changes to optimize performance. These changes with respect to operating a Finnigan Incos MSD include the following:

1. Adjusting the low resolution by keeping the V_{DC}/V_{RF} ratio and hence the slope constant but changing the magnitude of the RF envelope
2. Adjusting the high resolution by slightly changing the V_{DC}/V_{RF} ratio

TABLE 4.15 Applied V_{DC} and V_{RF} to the Pair of Quadrupole Rods for Six Snapshots in Time

Snapshot No.	$V(DC) + V(RF) \cos(\omega t)$	
	Rods 1 and 4	Rods 2 and 3
t_0	+20	-20
t_1	+140	-140
t_2	+20	-20
t_3	-100	+100
t_4	+20	-20
t_5	+140	-140

3. Adjusting the offset digital-to-analog converter by changing the zero reference of the RF start points
4. Adjusting the offset program by changing slopes while starting points are unaffected

47. IS THE MSD THE ONLY MASS SPEC USED IN TEQA?

No, a mass spec opposite in concept to the MSD yet utilizing similar principles is that of the quadrupole ion-trap mass spectrometer (ITD). The ITD is often found in environmental testing labs. The ITD is, theoretically, the three-dimensional analog of the MSD. It is generally less expensive than the MSD and is of a more recent development. If little to no DC voltage is applied to a quadrupole, ions with any m/z will remain in the $a-q$ stability region. The ion trap is depicted schematically in Fig. 4.44. The distance from the center to the end cap, z_0 , is called the axial distance. The distance from the center to the ring electrode r_0 , is called the radial distance. It helps to imagine that the plane of this paper contains the x, y coordinates and that this plane cuts through the ITD at its center. The z axis is then viewed as being above the $x-y$ plane and that the radial distance r rests within this plane. Ion traps are compact with the filament positioned just above the upper end cap in contrast to MSDs whereby the ion source is separated. A variable RF voltage is applied to the ring electrode while the two end cap electrodes are grounded or set at either a positive or negative voltage. As the RF voltage is increased, the heavier ions remain trapped while the lighter ions become destabilized and exit the trap through the bottom end cap. This bottom cap has openings drilled into the stainless steel to allow these ions to “escape.” A good introduction to ion-trap mass spectrometry including

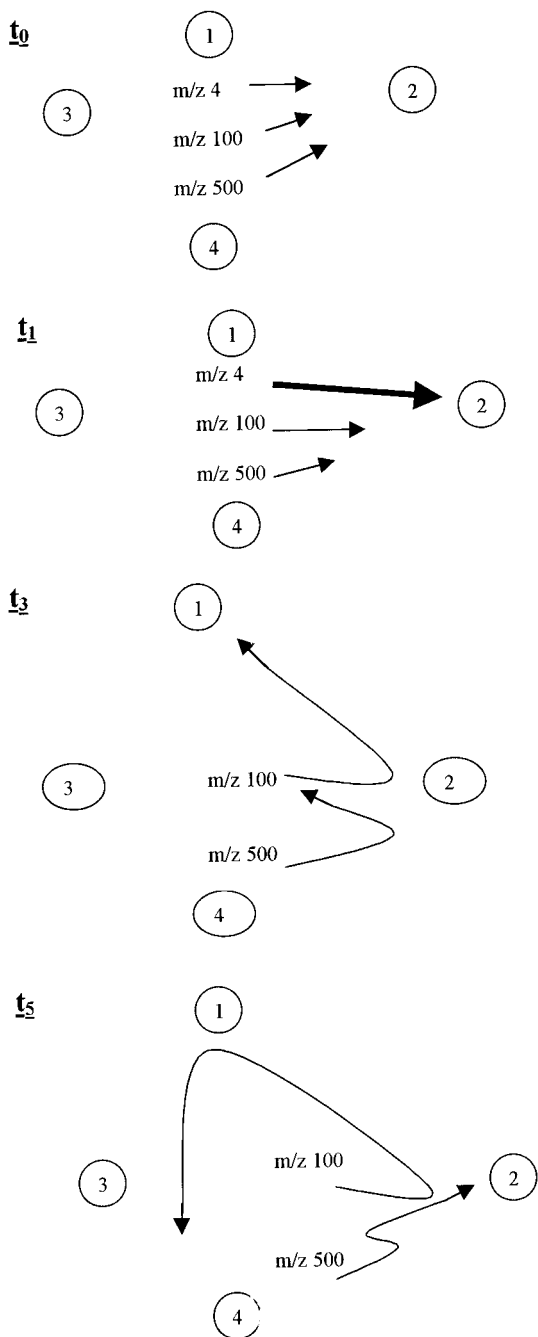


FIGURE 4.42 Behavior of three ions of different m/z ratios in the space between the four quadrupole rods.

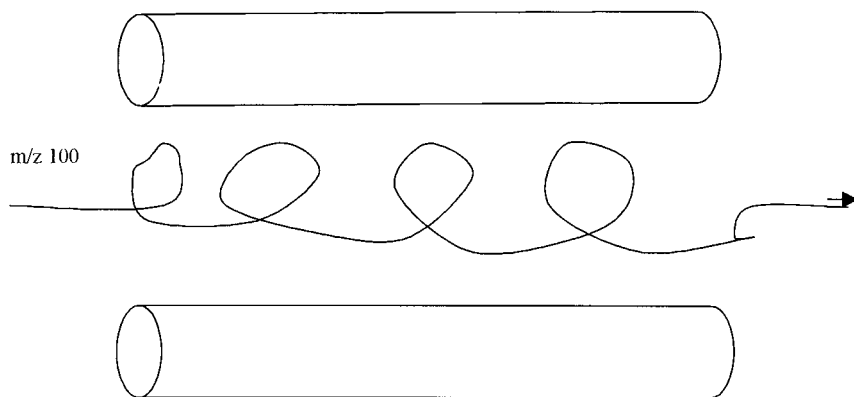


FIGURE 4.43 Schematic illustration of the somewhat helical trajectory taken by a given molecular ion or fragment ion of $m/z = 100$.

some interesting historical facts can be found in the article written by Cooks et al. (84).

With reference to Fig. 4.44, a symmetrical electrical field allows a consideration of only a radial and z displacement. The equations of motion of an ion in the three-dimensional quadrupole field are derived as in the case of the quadrupole mass filter:

$$\frac{d^2z}{dt^2} - \frac{4}{(m/e)r_0^2} [V_{\text{DC}} - V_{\text{RF}} \cos(\omega t)]z = 0$$

$$\frac{d^2r}{dt^2} + \frac{2}{(m/e)r_0^2} [V_{\text{DC}} - V_{\text{RF}} \cos(\omega t)]r = 0$$

With the following substitutions,

$$a_z = -2a_r = -\frac{16V_{\text{DC}}}{(m/e)(r_0^2)\omega_{\text{RF}}^2}$$

$$q_z = -2q_r = -\frac{8V_{\text{RF}}}{(m/e)(r_0^2)\omega_{\text{RF}}^2}$$

Let u represent either r or z and let $\xi = \omega t/2$.

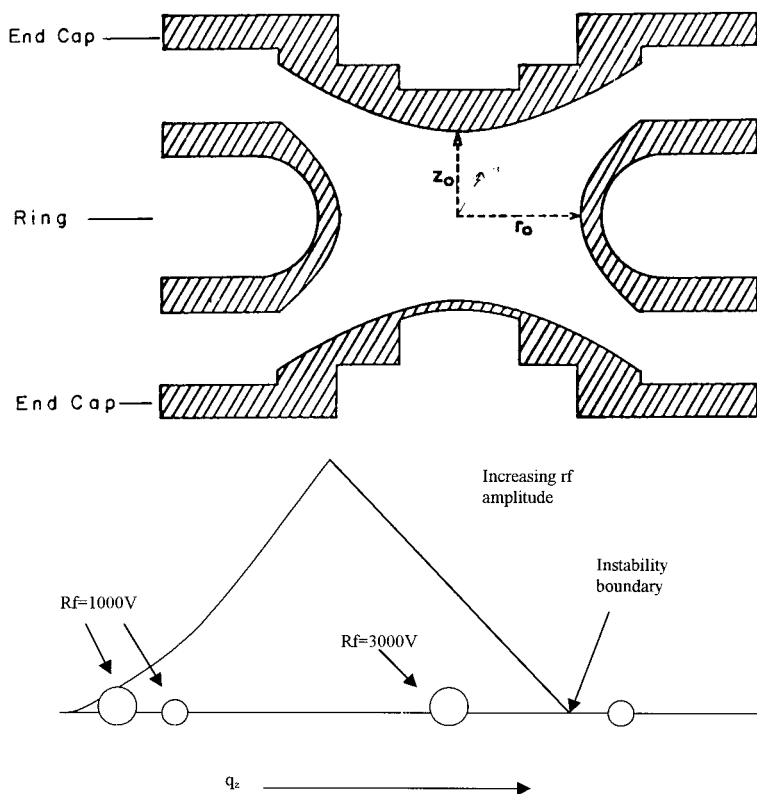


FIGURE 4.44 Schematic of a typical ion trap and a plot of a section of the ion trap near the origin illustrates how three ions of differing m/z ratios can be moved along the q_z axis.

In a manner similar to that derived for the MSD, the canonical form of the Mathieu equation results. Note that the stability parameters for the z and r directions differ by a factor of -2 . According to March and Hughes (85):

The stability regions defining the a , q values corresponding to solutions of the Mathieu equation that are stable in the z direction. The regions corresponding to solutions that are stable in the r direction must be thought of as being twice the size of the z -direction and then rotated about the q axis to account for the minus sign. The region of simultaneous stability comprises the intersection of these two regions.

Radial stability expressed in terms of a_r and q_r must also be maintained with z -direction stability. Ions of a given m/e are stable in the trap under operating conditions given in $a-q$ space. Figure 4.44 depicts a small section of the $a-q$ stability region very near to the origin for a typical ion trap. This figure graphically demonstrates how three ions of different m/e values can be moved along the q_z axis by simply increasing V_{RF} until the ions are, sequentially according to increasing m/e ejected from the trap into the instability region of $a-q$ space (84). This occurs at a value of $q_z = 0.908$.

48. AGAIN, THE MATH IS OK, BUT THIS TIME, WHAT ABOUT SOME APPLICATIONS OF C-GC-MS(ITD)?

This author has maintained a C-GC-MS(ITD) instrument for several years. One of the more interesting requests that not only utilized this instrument but also involved some sample preparation came from the forestry area. The homologous series of 2-aminoethanols (2-AEs) are organic compounds of importance to wood preservative treatments. Few methods if any can be found in the analytical literature that describe the qualitative and quantitative analyses of samples that contain any of the three 2-AEs. This series begins with 2-aminoethanol, followed by *N*-methyl-2-AE and, upon further substitution, *N,N*-dimethyl-2-AE. These compounds lack an UV-absorbing chromophore and, hence, are not directly amenable to analysis using HPLC. The 2-AEs are quite polar owing to the presence of both an amino functionality as well as being an alcohol. The C-GC-MS(ITD) that was configured in the author's laboratory is shown in Fig. 4.45. An Autosystem GC (Perkin-Elmer) was interfaced to a 800 Series (Finnigan, ThermoQuest) ITD. The WCOT column was passed through the transfer line into the ITD itself as is shown. The three 2-AEs were easily separated using a DB-624 WCOT (J & W Scientific). An analytical method was developed that included novel sample preparation techniques coupled to the separation by GC, identification, and quantitative analysis by the ITD (86).

49. WHERE DOES HPLC PLAY A ROLE IN TEQA?

This author was astonished one day back in the 1980s when he obtained a sample of an aqueous phase from a Superfund waste site that had been extracted (refer to the discussions of LLE in Chapter 3) with methylene chloride. The remaining "extracted" aqueous phase was sent to waste. The organic extract was analyzed using EPA Method 8270. This is a C-GC-MS(MSD) determinative method that separates, identifies, and

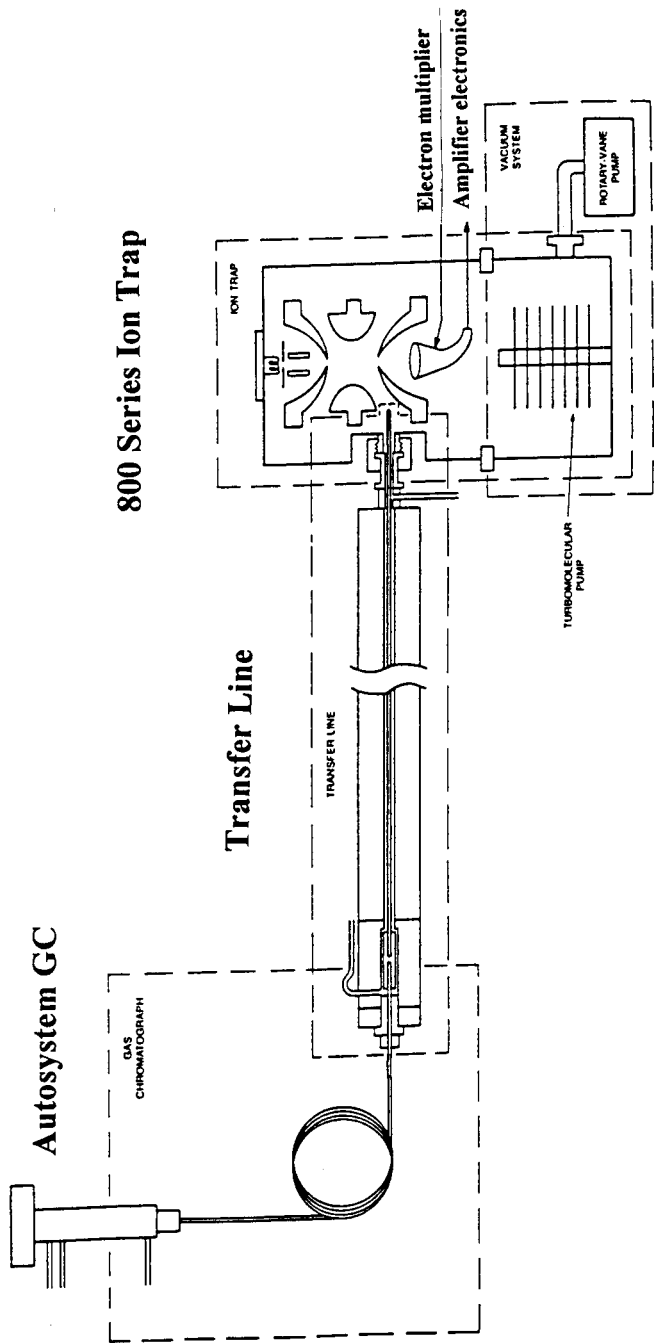


FIGURE 4.45 Configuration of a capillary GC-MS (ion trap) developed in the author's lab.

quantitates about 90 priority pollutant SVOCs. The author injected about 10 μL of this discarded aqueous phase into a HPLC in a reversed-phase mode using an UV-absorption detector. Over a half-dozen large peaks in the HPLC chromatogram were observed and the retention times for these peaks closely matched those of phenol and some substituted phenols! This author exclaimed, "I thought all of the priority pollutant organics were extracted out!" Upon further thought, if the percent recoveries of these phenols is low, then it is reasonable to assume that these polar analytes would remain in the aqueous phase due to the appreciable hydrogen-bonding between the hydroxy group on the phenol and the oxygen end of the water molecule. In Chapter 3, we discussed at length the fact that significant differences in partition coefficients between polar and nonpolar solutes leads to differences in percent recoveries. According to Fig. 4.1, if there remained residual phenols, then we should expect the complementary determinative technique of HPLC to detect these polar solutes!

Revision 4 of the SW-846 Methods, published in December 1996, lists nine specific EPA methods that use HPLC as the determinative technique. The kinds of SVOCs that require HPLC in the SW-846 methods provide some insights into just how HPLC complements GC with respect to TEQA. The following methods measure the SVOCs:

- Polycyclic aromatic hydrocarbons (PAHs)
- Carbonyl compounds such as aldehydes and ketones
- Acrylamide, acrylonitrile, and acrolein
- *N*-Methylcarbamates
- Solvent-extractable nonvolatile organic compounds
- Nitroaromatic and nitramines
- Tetrazene
- Nitroglycerine

Let us focus here on determining the various carbonyl compounds. Figure 4.46 shows a HPLC chromatogram for the separation and detection of the first six homologous series of aldehydes as their 2,4-dinitrophenylhydrazone derivatives accomplished in the author's laboratory. The reaction of aldehydes and ketones with 2,4-nitrophenylhydrazine under mildly acidic conditions in water to form stable hydrazones is a well-known reaction in organic chemistry. The homologous series with R representing C_1 , C_2 , C_3 , C_4 , C_5 , and C_6 react as follows:

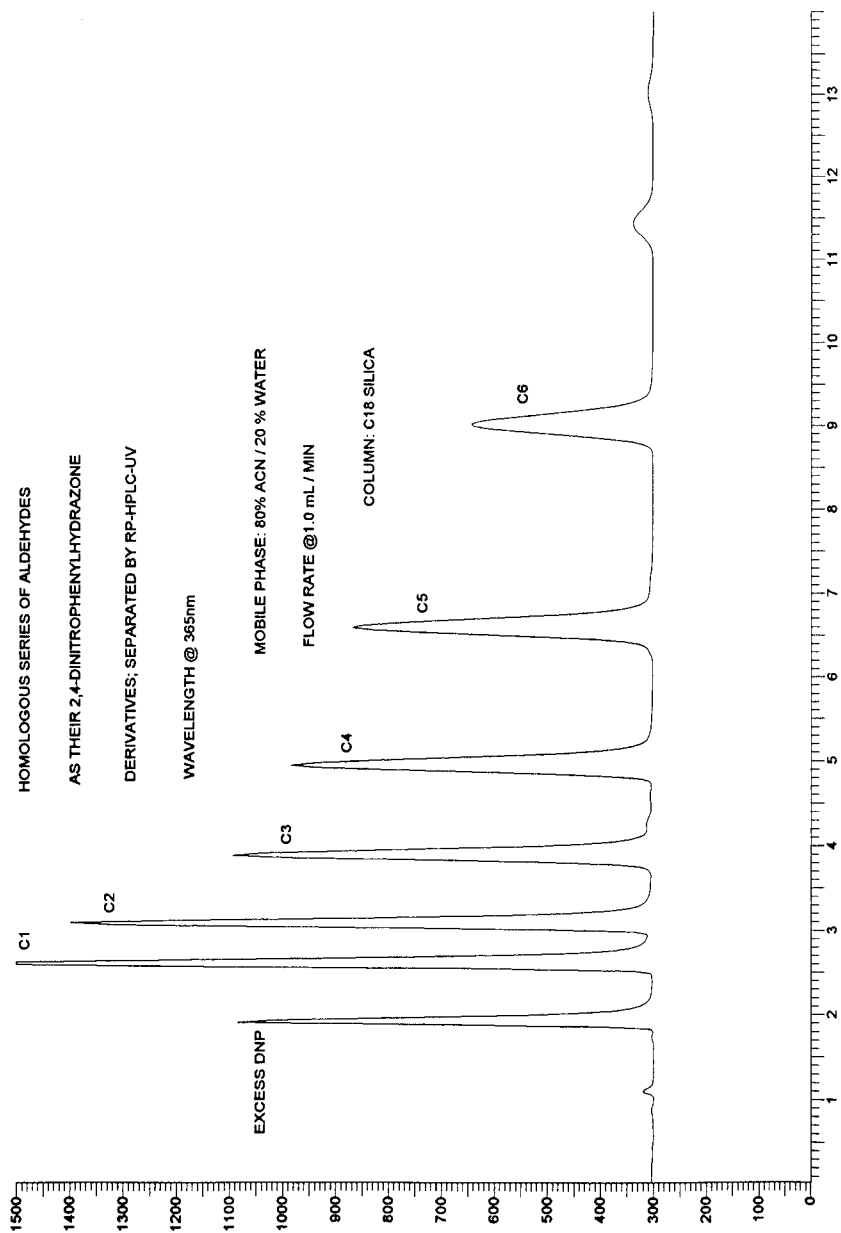
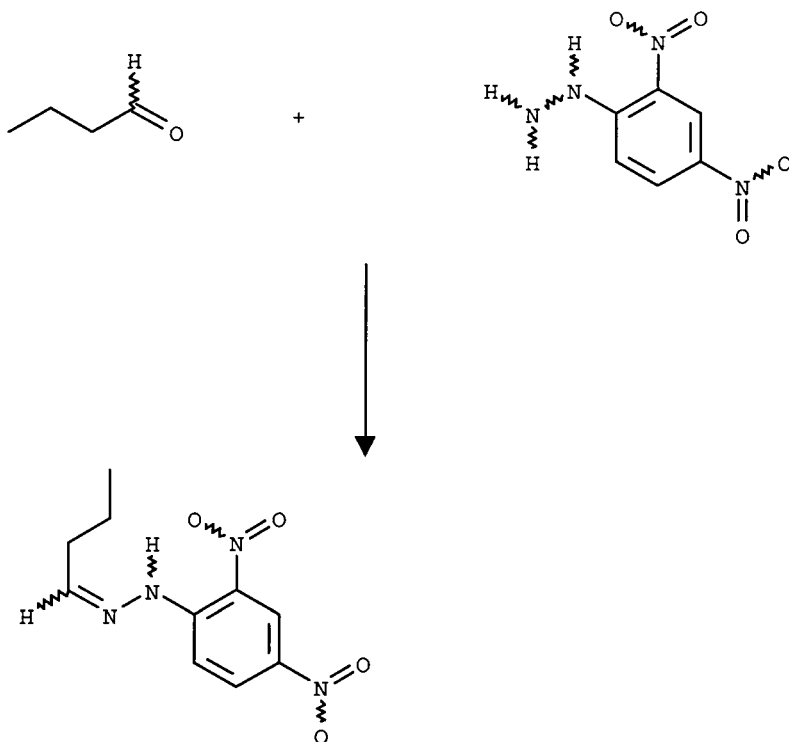


FIGURE 4.46 RP-HPLC separation and detection of the homologous series of aldehydes as their 2,4-dinitrophenylhydrazones.



2, 4-dinitrophenylhydrazone of an aldehyde

This method nicely illustrates the importance of HPLC to environmental testing labs and forms the basis of EPA Method 8315A “Determination of Carbonyl Compounds by HPLC” (87). Aldehydes and ketones are, in general, too polar to be isolated from aqueous matrices and are too thermally labile or thermally reactive for the GC determinative technique. Note the wavelength setting on the UV detector to 365 nm in Fig. 4.46. This wavelength is sufficiently removed from the crowded region between 200 and 300 nm and, therefore, can be considered somewhat unique for 2,4-diphenylhydrazones. Method 8315A combines both sample prep and determinative techniques and offers both LLE and SPE to isolate and recover the 2,4-diphenylhydrazones from an aqueous or soil matrix. The method suggests that gaseous stack samples are to be collected by Method 0011 and indoor air samples are to be collected by Method 0100. We now introduce the basics of the HPLC determinative technique.

50. WHAT IS HPLC?

Some nomenclature with respect to the broad field of liquid chromatography is in order. The concept of HPLC should be called high-performance liquid chromatography because column efficiency is so greatly improved when columns contained a stationary phase whose particle size has been reduced to 10 μm , or to 5 μm , and even as low as 3 μm ! This reduced particle size from that used in column liquid chromatography necessitates that the mobile-phase be pumped through these columns. The pressure drop across these low-particle-size columns required to achieve a flow rate that minimizes the height equivalent to a theoretical plate [refer to Eqs. (4.19) and (4.23)] exceeds 1000 psi and therefore necessitated pumping systems that could “push” liquids against this relatively high back-pressure. Instrumentation took what once was called low-pressure column chromatography and developed it into HPLC. However, the instrument itself is a high-pressure liquid chromatograph or aptly abbreviated as HPLC, hence the source of confusion in describing this important determinative technique.

A block diagram of a high-pressure liquid chromatograph is shown in Fig. 4.47. This diagram is a useful way to understand how an HPLC works and shows how HPLC is clearly a modular instrument. This modular nature of HPLC enables a number of options to be considered as introduced in Fig. 4.47. The reservoir can be either left at atmospheric pressure or pressurized with inert gas. However, a mobile-phase that is not pressurized must be degassed, otherwise air bubbles are continuously “squeezed” out of the mobile-phase and accumulate in the micro flow cell of the detector. These trapped air bubbles prevent the analyst from obtaining a stable detector baseline. This is particularly true for a UV-absorbance detector. Pump systems can be either those that deliver essentially a constant flow rate or those that deliver a constant pressure. Reciprocating piston pumps deliver a constant flow rate and are manufactured with single, dual, or even triple pump heads. Pumps designed for HPLC must be able to withstand fluid pressure in excess of 6000 psi! A common safety feature built in to most HPLC pumps is a maximum pressure sensor. In practice, this value is set at approximately 4000 psi. If the back-pressure at the column head exceeds this value, the pump will shut down automatically. The addition of a pressure transducer and a pulse dampener completes the necessary accessories for a good pump. Gradient elution HPLC is analogous to temperature programming in GC and is achieved by varying the percent organic modifier in the mobile-phase after the sample has been injected. There are two different designs for performing gradient elution. One is to mix two or more

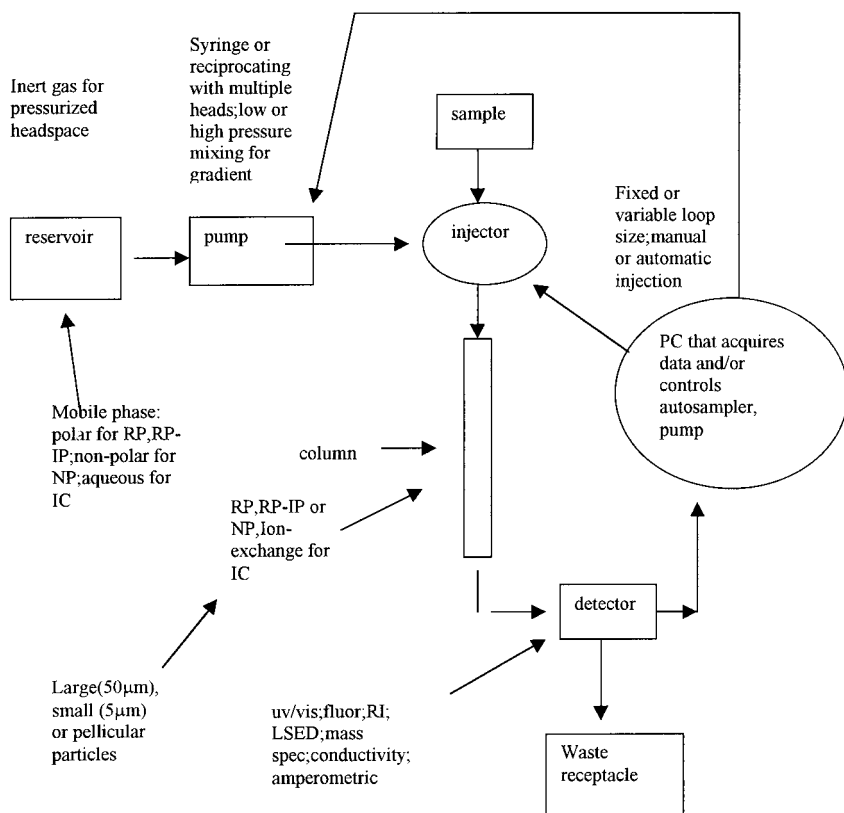


FIGURE 4.47 Block diagram of a high-performance liquid chromatograph.

different solvents at low pressure and the other is to mix them at high pressure.

One of the great developments that led to an increase in the scope of HPLC occurred with the preparation of chemically bonded silicas. In addition, it was the realization that a reduction in particle size along with an increase in particle size homogeneity would lead to highly efficient columns. Together, these bonded-phase silicas led to the development of reversed-phase HPLC (RP-HPLC) and thus greatly expanded the scope of this instrumental technique. Figure 4.1 shows that for nonvolatile organics that are somewhat polar, RP-HPLC is most appropriate for their retention and separation. The fact that the analyst can make a direct aqueous injection into a HPLC that is operated under reversed-phase conditions enabled

RP-HPLC to take on a major role as a determinative technique in TEQA. Snyder and Kirkland, in their classic book on HPLC, commented on how “modern LC arose” by stating the following (88):

Modern LC is based on developments in several areas: equipment, special columns and column packings, and theory. High-pressure, low-dead-volume equipment with sensitive detectors plays a vital role. The new column packings that have been developed specifically for modern LC are also essential for rapid, high-performance separations. Theory has played a much more important role in the development of modern LC than for preceding chromatographic innovations. Fortunately, the theory of LC is not very different from that of GC, and a good understanding of the fundamentals of GC had evolved by the early 1960s. This GC theory was readily extended to include LC, and this in turn led directly to the development of the first high-performance column packings for LC and the design of the first modern LC units The potential advantages of modern LC first came to the attention of a wide audience in early 1969 However, modern LC had its beginnings in the late 1950s, with the introduction of automated amino acid analysis by Spackman, Stein, and Moore and this was followed by the pioneering work of Hamilton and Giddings on the fundamental theory of high-performance LC in columns, the work of C. D. Scott at Oak Ridge on high-pressure ion-exchange chromatography, and the introduction of gel permeation chromatography by J. C. Moore and Waters Associates in the mid-1960s.

51. HOW DID THE NAME “REVERSED-PHASE” HPLC COME ABOUT?

A nonpolar mobile phase passing through a packed column that contains a polar stationary phase defines normal-phase HPLC (NP-HPLC). For example, if *n*-hexane comprises the mobile-phase and silica gel is used for the stationary phase, separations of nonpolar organic analytes as shown in Fig. 4.1 is accomplished. With respect to neutral organic compounds, the polar and ionic domains cannot be reached by NP-HPLC. NP-HPLC was the first high-pressure form of liquid chromatography to be developed. If the stationary phase could be made hydrophobic by chemical treatment and the mobile phase made more polar, a “reversal” of mobile/stationary-phase polarities could be achieved. Like it or not, we are stuck with this nomenclature! RP-HPLC has certainly extended the range of analyte polarity that

can be separated and detected! Reversed-phase ion-pair HPLC (RP-IP-HPLC) has enabled organic cations such as quaternary ammonium salts and organic anions such as anionic surfactants to be retained, separated, and quantitated, thus extending the range of analyte polarity even further! To begin to realize the influence of mobile-phase composition on HPLC resolution, let us return to the fundamental resolution equation.

52. WHY DOES THE MOBILE-PHASE EXERT SO MUCH INFLUENCE ON R_S ?

We discussed Eq. (4.30) earlier with respect to GC theory. This equation applies equally well to HPLC. Unlike GC, the HPLC mobile phase exerts considerable control over the chromatogram and this is evident in the chromatograms shown in Fig. 4.48. Consider an initial injection of two poorly resolved peaks such as in Fig. 4.48A. The capacity factor can be varied as shown in Fig. 4.48B and the result either decreases R_S when k' is decreased or increases R_S when k' is increased as shown. However, sometimes an increase in k' also leads to increased peak broadening and hence to no greater advantage with respect to R_S as shown. The column can be made more efficient by changing the stationary phase as shown in Fig. 4.48C. The chemical nature of the stationary phase can be changed to make the column more selective and thus serve to increase α , as is shown in Fig. 4.48D. To answer the question, the magnitude of the partition coefficient, K , for a neutral organic compound between a liquid mobile phase and a given stationary phase can be significantly changed by a change in the percent organic modifier. Equation (4.31) suggests that significant changes in K leads to differences in k' . As is shown in Fig. 4.49, an increase in the percent acetonitrile (solvent B by convention) from 43% in distilled deionized water (solvent A) to 50% acetonitrile in acetate buffer at pH 5.0 not only reduced k' for 3-(trifluoromethyl)-*p*-nitrophenol (TFM) but also improved peak shape. A well-known relationship in RP-HPLC relates the capacity factor in a given organic/aqueous mobile phase k to the capacity factor in 100% water, k_w , and the volume fraction of organic in the mobile phase ϕ , where $\phi = \% \text{ B}/100$ according to (89)

$$\log k = \log k_w - S\phi$$

For low-molecular-weight compounds, $S \approx 4$. Thus, k increases by a factor of 2–3 for a decrease of 10% B. Three RP-HPLC chromatograms from the author's laboratory are shown Fig. 4.50. The software enables all three chromatograms to be “stacked” as shown. The top chromatogram shows a column that has deteriorated beyond its useful life. The middle chromato-

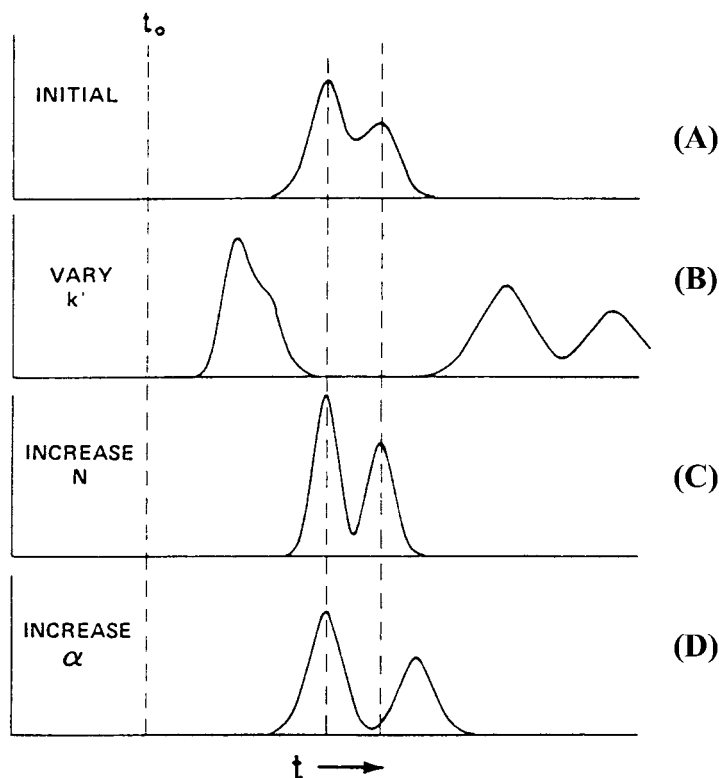


FIGURE 4.48 The effect on chromatographic resolution of changes in k' , N , or α .

gram illustrates how a change in the column can radically improve HPLC column efficiency. The isocratic test mix available from Supelco consists of a homologous series of substituted parabens (esters of *p*-hydroxybenzoic acid). The bottom chromatogram shows how the injection of the same mixture using the same column looks when the % B is lowered. When viewing Fig. 4.50, note that the time scales have not been aligned.

53. ARE ALL OCTADECYL-BONDED SILICA HPLC COLUMNS THE SAME?

The stacked HPLC chromatograms shown in Fig. 4.51 should provide the answer to this question. Both chromatograms were obtained on the same instrument, using the same mobile-phase, however the octadecyl-bonded

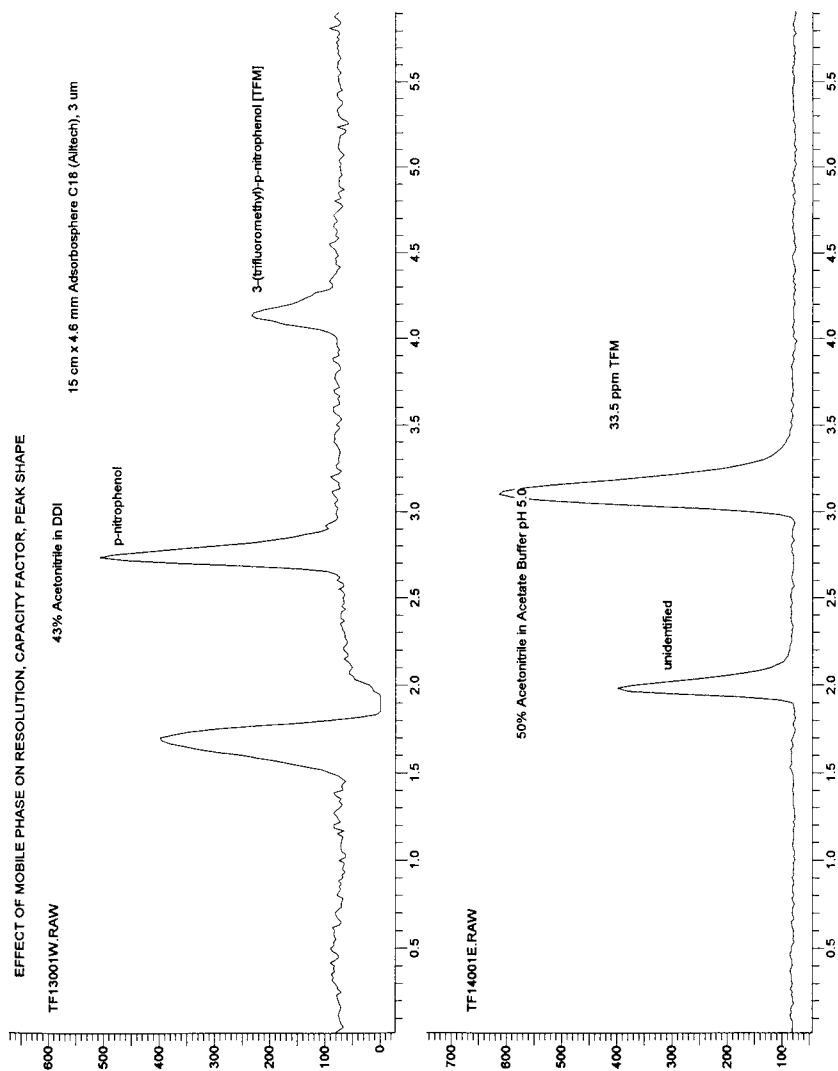


FIGURE 4.49 Effect of mobile phase on R_S , k' , and peak shape in RP-HPLC.

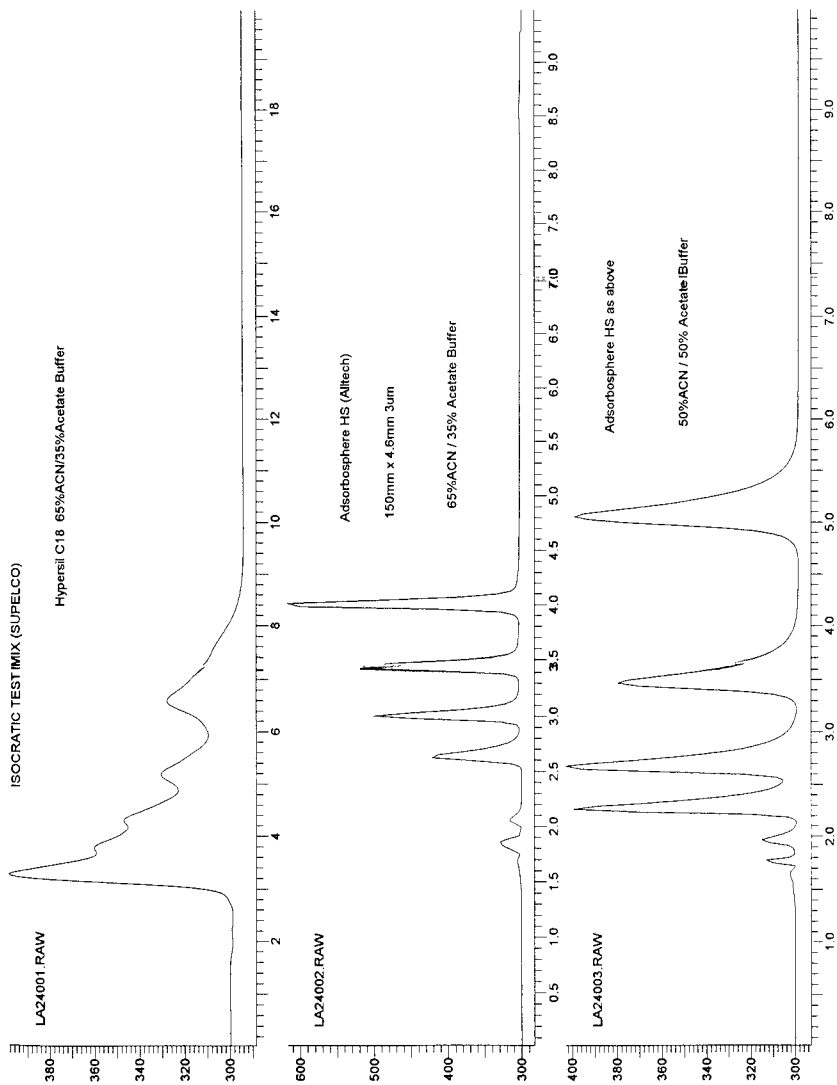


Figure 4.50 Comparison of the RP-HPLC chromatograms for a deteriorated column versus an efficient column.

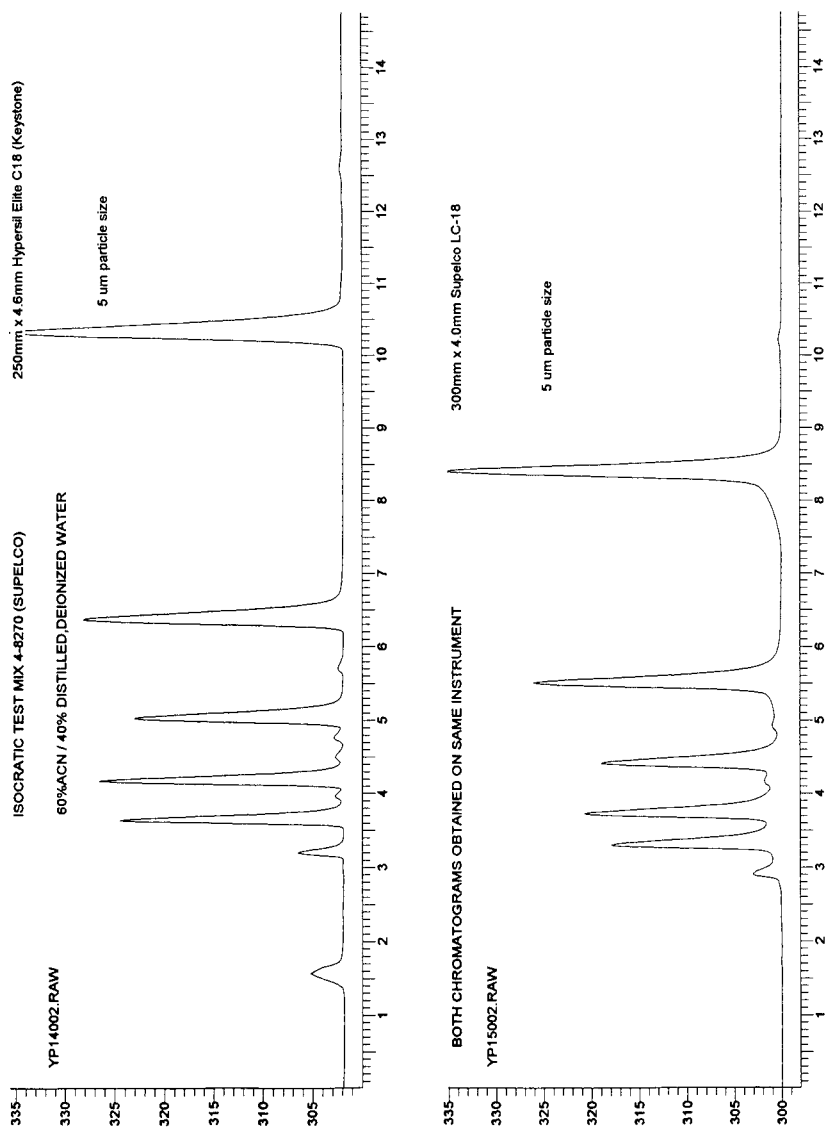


FIGURE 4.51 Comparison of the RP-HPLC chromatograms between two C₁₈ columns from two different manufacturers.

silica (ODS) columns used were obtained from two different suppliers as indicated. Note that the column dimensions differ slightly between both columns. Interpretation of the top chromatogram indicates that this column exhibits a much higher k' when compared to the column used to generate the lower chromatogram. A narrower peak width is, however, evident in the bottom chromatogram. The number of theoretical plates for an HPLC column can be calculated using either Eq. (4.21) or (4.22) depending on how the peak width is measured. When stating how “good” a column is, Snyder et al. have listed the following requirements (90):

- Plate number N for a given value of k'
- Peak asymmetry
- Selectivity, α , for two different solutes
- Column back-pressure
- Retention, k' , reproducibility
- Bonded-phase concentration
- Column stability

They also suggest that the following equation be used to estimate the column plate number for small molecules under optimum conditions for a column length L and a particle diameter d_p according to (90)

$$N \approx \frac{3500L(\text{cm})}{d_p (\mu\text{m})}$$

As is quite evident from interpreting the chromatograms in Fig. 4.50, RP-HPLC columns have a finite lifetime! It is good practice for the analyst to keep a record of N (as calculated using the above equation) versus either time or number of injected samples in an attempt to continuously monitor column performance. Because HPLC reciprocating pumps maintain constant flow rate, a continuous observation of the back-pressure or pressure buildup at the front on the HPLC column is an important parameter to monitor. Making sure that there are no leaks in an operating HPLC is also very important.

Another useful equation that is used to predict the back-pressure or pressure drop, ΔP , for well-packed columns having similar operating conditions where the mobile-phase viscosity η in centipoise for a dead time t_0 for columns packed with spherical particles can be found from (90)

$$\Delta P(\text{psi}) = \frac{3000L(\text{cm}) \eta(\text{cP})}{t_0 d_p^2 (\mu\text{m})}$$

Columns packed with irregular particles might yield back-pressures that are higher. A new spherical-particle column should have a ΔP no greater than about 30% in excess of that predicted by the above equation. Let us return to Fig. 4.47 and take a brief look at some HPLC detectors.

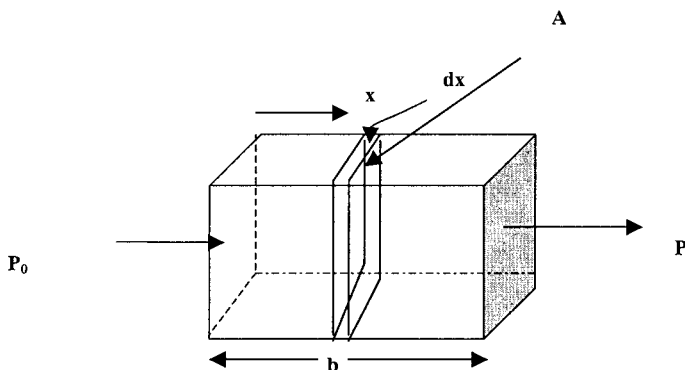
54. HOW DO HPLC DETECTORS WORK?

Depicted in Fig. 4.47 are the names of the major types of HPLC detectors. It has been said that the FID is to the GC, what the UV-vis-absorbance detector is to the HPLC! However, unlike GC, there is no universal HPLC non-mass-spectrometric detector! The recent advances in LC-MS have made this hyphenated technique a universal one. Because this technique has not as yet made significant “in roads” into TEQA, we will not, therefore, discuss LC-MS here. EPA Method 8321A “Solvent Extractable Nonvolatile Compounds by High Performance Liquid Chromatography/Thermospray/Mass Spectrometry or Ultraviolet Detection” and Method 8325 “Solvent Extractable Nonvolatile Compounds by High Performance Liquid Chromatography/Particle Beam/Mass Spectrometry” provide the first methods of relevance to TEQA in this regard (87). We will focus on two detectors designed for HPLC that are in current use in the author’s laboratory.

The first is a UV-vis-absorption or absorbance spectrophotometric (the phenomenon responsible for the signal is absorption, however, what is actually measured is absorbance, UV) detector. The second is a molecular fluorescence luminescence spectrophotometric (commonly called fluorescence, FL) detector. Other non-mass-spectrometric detectors designed for HPLC include refractive index (RI), electrochemical (EC), and of a more recent vintage, the light-scattering evaporative detector (LSED). RI and LSED HPLC detectors are not sensitive enough to meet the needs of TEQA. Electrochemical HPLC detectors have the required sensitivity, but due to frequent fouling of electrode surfaces they have not really found a place in TEQA. This author knows of no EPA methods as yet that incorporate EC HPLC detectors. For this reason, EC HPLC detectors will be not considered.

Organic compounds that possess an ultraviolet- or visible-absorbing chromophore obey the Beer-Lambert or Beer-Bouguer law of spectrophotometry. In what is generally termed molecular absorption spectrophotometry, a cuvette (in the case of stand-alone UV-vis spectrophotometers) or a micro-flow cell (in the case of flow through HPLC UV-vis detectors). We now proceed to derive the fundamental equation that relates absorbance as measured on an UV-vis HPLC detector to concentration because this relationship is important to the practice

of TEQA. Similar approaches have been shown in two of the most popular textbooks in analytical chemistry (91). Let us start by considering what happens when UV radiation impinges onto a cuvette of path length b :



Ultraviolet or visible radiation incident on the infinitesimal volume of area A and thickness dx experiences a decrease in transmitted intensity or power dP that is proportional to the incident power P , to the number of absorbing molecules Nc , where N is Avogadro's number and c is the concentration of absorbing species in moles per liter, and to the thickness dx according to

$$dP = -\beta P N c A dx$$

where β is a proportionality constant. This equation can be rearranged and integrated as follows:

$$-\frac{dP}{P} = \beta c dx$$

$$-\int_{P_0}^P \frac{dP}{P} = \beta c \int_0^b dx$$

At $x = 0$, $P = P_0$, and at $x = b$, $P = P$, and we have the limits of integration that are necessary to evaluate these integrals. Rearranging and removing the negative sign yields the desired outcome:

$$\ln\left(\frac{P_0}{P}\right) = \beta cb$$

Converting from the natural to the common logarithm while substituting $\ln x = (\ln 10)(\log x)$ yields

$$\log\left(\frac{P_0}{P}\right) = \left(\frac{\beta}{\ln 10}\right) cb$$

The absorbance, A , is defined as the logarithm of the ratio of the incident intensity, P_0 , to the transmitted intensity, P , so that

$$A = \epsilon bc$$

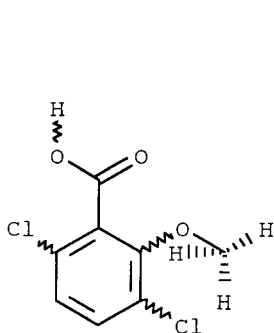
This equation states that the absorbance is directly proportional to solute concentration for a given solute/solvent, (i.e., ϵ , the molar absorptivity) and for a fixed path length b . It must be remembered that the absorbance and the molar absorptivity are dependent on the wavelength. It becomes important in practice for an analyst to know how the molar absorptivity varies with wavelength, λ . The percent transmittance, $\%T = 100T$, a common term used with stand-alone spectrophotometers, can be related to the absorbance by manipulating the above definition for A according to

$$\begin{aligned} A &= \log\left(\frac{P_0}{P}\right) = -\log\left(\frac{P}{P_0}\right) \\ &= -\log\left(\frac{\%T}{100}\right) \\ &= 2 - \log(\%T) \end{aligned}$$

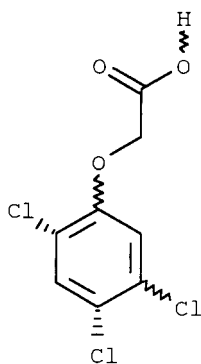
It is seen from this relationship that when no radiation is transmitted, $\%T = 0$ and $A = 2$, whereas if all incident radiation is transmitted through the cuvette or micro-flow cell, the $\%T = 100$, all radiation is transmitted, and $A = 0$. The simple proportion between A and c forms the basis for TEQA using UV-vis-absorption HPLC detectors.

55. HOW DO YOU GO ABOUT DOING QUANTITATIVE ANALYSIS USING HPLC WITH UV DETECTION?

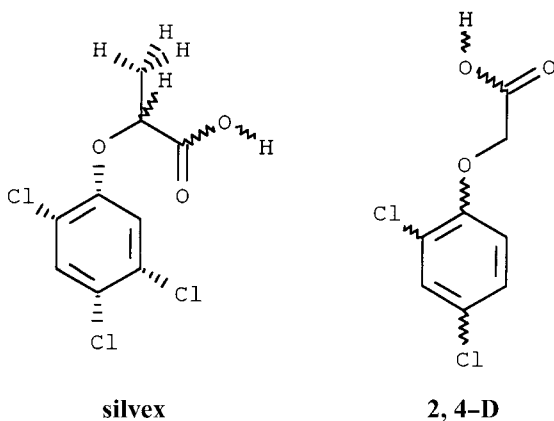
Because a solute's absorbance, A , and its molar absorptivity, ϵ , depend on the wavelength of the incident UV radiation, it is necessary that a wavelength be found that maximizes ϵ . It becomes important, then, to either know the UV absorption spectrum for the analyte of interest or to have some means to record the UV absorption spectrum. This can be accomplished using a stand-alone scanning UV-vis spectrophotometer. An absorption spectrum is a plot of absorbance against wavelength across either the UV alone (200–400 nm) or the UV and visible (200–700 nm). Ultraviolet-absorption spectra, unlike mass spectra, are relatively uninformative. Absorption of UV photons is enough not only to excite electrons from the ground electronic state to the first excited but also to excite rotational and vibrational quantized levels within each electronic state. This yields UV absorption that covers a wide range of wavelengths and hence leads to large absorption bands. An experiment in Chapter 5 provides a number of practical details related to molecular absorption spectra. The chlorophenoxy acids provide a good illustration. These organic acids are used as herbicides and are likely found in the environment. Dicamba, 3,6-dichloro-2-methoxybenzoic acid, although not a phenoxy acid, is included along with 2,4-dichlorophenoxyacetic (2,4-D) and two structurally very similar chlorophenoxy acids, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2-(2,4,5-trichloro)-propionic acid commonly called Silvex. Molecular structures for all four compounds are as follows:



Dicamba



2, 4, 5-T



The UV-absorption spectra were recorded at the apex of the chromatographically resolved peak using RP-HPLC with a photodiode-array UV detector. This detector, unlike a fixed-wavelength detector, can record all absorbances throughout the UV region of interest simultaneously. All four compounds yield UV-absorption spectra that show intense absorbance below 250 nm while having an absorption peak maximum between 270 and 295 nm. The other factor to consider in choosing the most sensitive wavelength for trace analysis is the UV cutoff of the organic modifiers used in the mobile-phase. A selected list of solvents is given in Table 4.16 (92). The UV cutoff is the wavelength above which useful quantitative analysis can be obtained.

The solvent viscosity, boiling point, and solvent polarity are all important factors in arriving at the optimum organic solvent to be used in the mobile-phase. For example, acetone is a low-viscosity liquid with a boiling point that is appropriate for RP-HPLC and a polarity that when mixed with water yields a mobile-phase with sufficient solvent strength that would enable moderately polar solutes to have k' values between 2 and 10. However, its UV cutoff is so high as to render the solvent useless for TEQA based on molecular absorption in the low-UV region where most organic compounds absorb! Acetonitrile, on the other hand, has similar physical properties combined with a low-UV cutoff. Cyclohexane has a favorable UV cutoff, viscosity, and boiling point. However, its polarity is so low that it is not even miscible in water! Cyclohexane is useless as an organic modifier for RP-HPLC, yet is appropriate as a mobile-phase additive in normal-phase (NP)-HPLC due to the extremely low solvent polarity. Acetonitrile, methanol, and tetrahydrofuran are the most commonly used organic modifiers for the laboratory practice of RP-HPLC, whereas cyclo-

TABLE 4.16 Physico-chemical Properties of Common Organic Solvents, Including Water

Solvent	UV cutoff (nm)	Viscosity (cP)	Boiling point (°C)	Polarity P'
Acetone	330	0.36	56.3	5.1
Acetonitrile	190	0.38	81.6	5.8
Cyclohexane	200	1.0	80.7	0.2
Ethyl acetate	256	0.45	77.1	4.4
Hexane	195	0.31	68.7	0.1
Methanol	205	0.55	64.7	5.1
Methylene chloride	233	0.44	39.8	3.1
Tetrahydrofuran	212	0.55	66.0	4.0
Water	190	1.00	100.0	10.2

hexane and *n*-hexane are the most common solvents for NP-HPLC. Let us consider the inner workings of a typical UV-absorbance HPLC detector. NP-HPLC is of limited value to TEQA and will not be considered any further. Because this book emphasizes quantitative measurement, we next focus on the HPLC-UV detector.

56. HOW DOES THE ABSORBANCE OF A SOLUTE GET MEASURED?

The optics for the Model 2487[®] dual λ Absorbance Detector (Waters) are shown in Fig. 4.52. A deuterium lamp is the source of UV radiation and the ellipsoidal mirror collects light from the lamp and focuses it through the filter wheel onto the entrance slit. The spherical mirror directs light toward the grating. Another part of the spherical mirror focuses dispersed light of a particular wavelength, determined by the grating angle, onto the entrance of the flow cell. Prior to the flow cell, a portion of the band is split to a reference photodiode while the remaining incident intensity is transmitted through the flow cell to the sample photodiode. The preamplifier board integrates and digitizes the currents from the photodiodes for processing by the signal processing electronics and output to a computer. When a new wavelength is entered through the front panel, the detector rotates the grating to the appropriate position. The difference between a conventional flow cell and the TaperSlit[®] is shown in Fig. 4.53. According to Waters, the TaperSlit flow cell renders the detector insensitive to changes in mobile phase refractive index. This is significant in gradient elution HPLC where the mobile-phase composition changes with time. As shown in Fig. 4.53, this tapered design minimized the amount of radiation that is reflected off of the inner wall, thus allowing a greater UV light throughput. This is not the only non-mass-spectrometric HPLC detector useful to TEQA. Let us consider measuring the UV-induced fluorescence, instead of measuring UV absorption.

57. WHAT IS A HPLC FLUORESCENCE DETECTOR?

The fluorescence HPLC detector (HPLC-FL) is similar to the UV-absorption HPLC detector (HPLC-UV) in that a source of UV radiation is made incident to a micro-flow cell in which the chromatographically resolved analyte passes through. However, a photomultiplier tube (PMT) and associated optics are positioned at right angles relative to the incident UV radiation. Lakowicz has articulated just what molecular luminescence is (93):

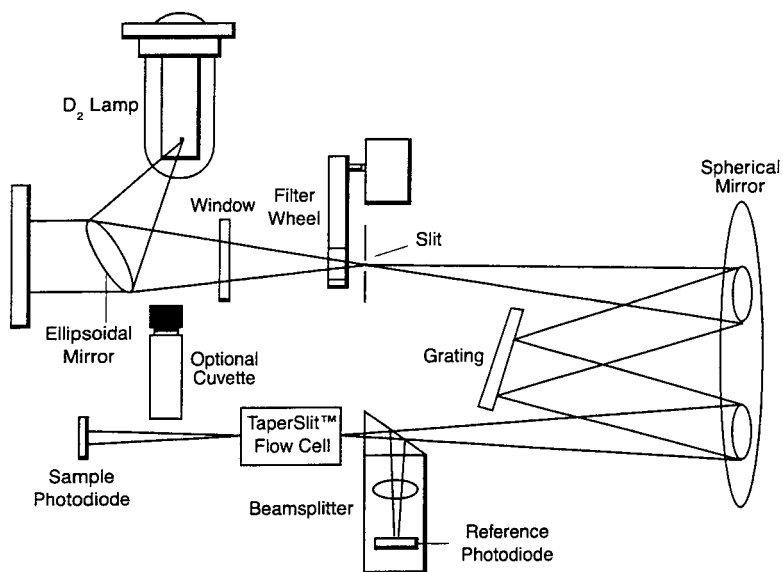


FIGURE 4.52 Optics diagram for the Model 2487[®] (Waters) UV absorbance HPLC detector.

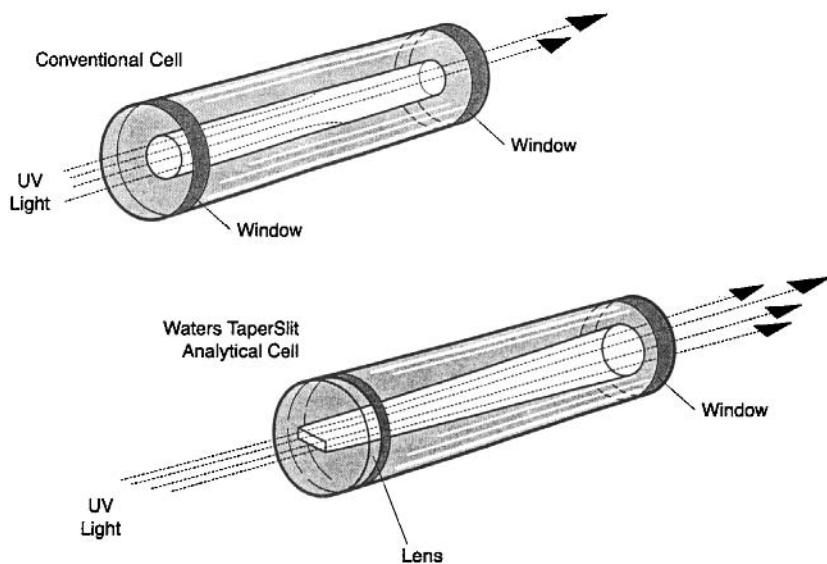
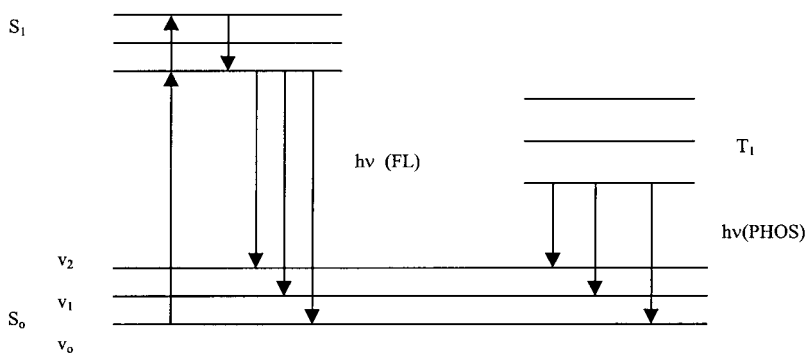


FIGURE 4.53 Comparison between a conventional and TaperSlit[®] (Waters) HPLC flow cell.

Luminescence is the emission of photons from electronically excited states. Luminescence is divided into two types, depending upon the nature of the ground and the excited states. In a singlet excited state, the electron in the higher-energy orbital has the opposite spin orientation as the second electron in the lower orbital. These two electrons are said to be paired. In a triplet state, these electrons are unpaired—that is, their spins have the same orientation. Return to the ground state from an excited singlet state does not require an electron to change its spin orientation. A change in spin orientation is needed for a triplet state to return to the singlet ground state. Fluorescence is the emission that results from the return to the lower orbital of the paired electron. Such transitions are quantum mechanically “allowed”. . . . Phosphorescence is the emission that results from transition between states of different multiplicity, generally a triplet state returns to a singlet ground state.

Ewing defines molecular luminescence that includes fluorescence, phosphorescence, and Raman spectroscopies as methods whereby radiation absorbed by molecular species are reemitted with a change in wavelength (94).

Both organic and inorganic compounds exhibit fluorescence. Molecules that display significant fluorescence possess delocalized electrons due to conjugated double bonds. A fluorescence spectra is a plot of FL emission intensity versus wavelength of the emitted radiation. A simplified Jablonski diagram provides a qualitative explanation of how fluorescence (FL) and phosphorescence (PHOS) spectra originate:



There exists within molecules a myriad of quantized vibrational levels in both the ground and first excited electronic state. Not shown in the dia-

gram are the myriad of rotational quantized levels within each vibrational level. Transitions as shown in the diagram yield absorption and fluorescence spectra that are quite broad. The fact that these spectra are obtained for solutes that are dissolved in solvents is also a contributor to broad bands. Photons emitted due to $h\nu(\text{FL})$ are on the order of nanoseconds, whereas photons emitted due to $h\nu(\text{PHOS})$ range from milliseconds to seconds.

Fluorescence quenching is the term used to explain any decrease in the analyte emitted fluorescence intensity due to the influence of the sample matrix. Quenching can also be attributed to the analyte itself and is called self-quenching. Ewing discusses the effect of quenching when the concentration of phenol dissolved in water is increased beyond 10 ppm with a maximum in the emitted intensity at around 75 ppm, followed by a gradual loss in intensity due to self-quenching (95). A quenching agent, if present, contributes to a loss in the emitted intensity. A good example is the quenching of PAHs by dissolved oxygen.

58. HOW IS IT THAT FLUORESCENCE CAN BE QUANTITATED?

For a single fluorophore, as would be the case for a chromatographically resolved compound in the HPLC chromatogram, the fluorescence is related to the incident UV radiation of power P_0 according to

$$F = P_0 K (1 - 10^{-A})$$

where A is the UV absorbance and K is a constant for a given system and instrument and is related to the quantum yield. This expression can be made more useful by considering a Taylor's series expansion for the exponential term:

$$F = P_0 K \left(2.30A - \frac{(2.30A)^2}{2!} + \frac{(2.30A)^3}{3!} + \dots \right)$$

To a first approximation, for solutions of low absorbance that are most likely the case for TEQA, the higher-order terms can be neglected so that

$$F \approx P_0 K (2.30)A$$

We see that a direct proportionality exists between the emitted fluorescence intensity denoted by F and the sample absorbance denoted by A . If a stand-alone spectrofluorimeter is used to conduct a quantitative analysis, the error introduced by neglecting the A^2 term should be considered. However, for a fluorescence HPLC detector where a set of working calibration standards are used (refer to Chapter 2) and where the concentration of fluorophore is relatively low, it is sufficient to ignore the higher-order terms. Upon substituting for A , we obtain

$$F \approx P_0 K(2.30)(\epsilon bc)$$

$$\approx K' \epsilon bc \approx K'' c$$

The linear dynamic range of this detector is limited at the high end by fluorescence quenching due to self-absorption. These equations demonstrate that a fluorescence HPLC detector is intrinsically more sensitive than an UV absorption HPLC detector because for dilute solution, the PMT senses a faint light against a dark background. For an UV absorption detector, the PMT or photodiode compares a slightly lower transmitted intensity P against a higher incident intensity P_0 , and this small difference is related to analyte concentration.

Instrumentation to measure the fluorescence of a substance can be as simple as a filter fluorimeter or as complex as a dual excitation/emission spectrofluorimeter! A schematic of the Model 474[®] (Waters) dual monochromatic fluorescence HPLC detector is shown in Fig. 4.54. A xenon lamp is the source of UV radiation and this light reflects off of a mirror to an excitation grating through a narrow entrance slit. This monochromator enables a narrow band of excitation wavelengths to be selected. The selected UV photons are passed through a beam splitter whereby a portion of this excitation radiation is detected by a photodiode. This photodiode enables corrections to the lamp output to be made. The collection mirror is positioned at a right angle with respect to the direction of the excitation light, as shown in Fig. 4.54. The fluorescence radiation is collimated and reflected through a narrow emission slit where it is diffracted off of the grating. This emission monochromator selects a narrow band of fluorescence wavelengths whose intensity reaches a PMT. The output of the PMT is amplified and fed to an analog-to-digital (A/D) converter and then fed into a PC for processing and quantitative analysis. Contemporary fluorescence HPLC detectors like the Model 474 are completely microprocessor controlled and this is best shown in Fig. 4.55.

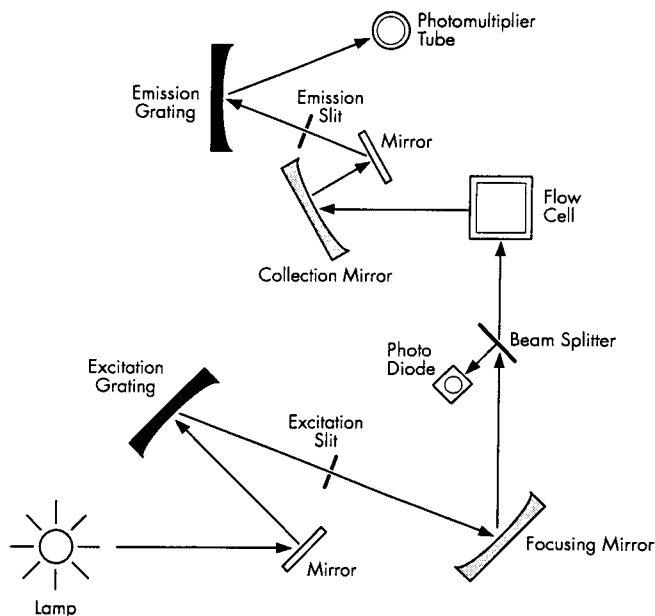


FIGURE 4.54 Schematic of the Model 474[®] (Waters) fluorescence HPLC detector.

59. HOW DO UV ABSORPTION AND FLUORESCENCE EMISSION HPLC CHROMATOGRAMS COMPARE?

The answer lies in the nature of the chemical compounds that are of interest. For example, there is little difference in HPLC detector sensitivity between UV absorption and fluorescence emission for phenol, but a great difference exists for naphthalene. Naphthalene is a fused aromatic and allows for extensive delocalization of electrons and is quite sensitive when measured by RP-HPLC-FL. For both analytes of environmental concern being dissolved in water or dissolved in the RP-HPLC mobile-phase, a vivid illustration of this difference in HPLC detector sensitivity is shown in Fig. 4.56. A RP-HPLC-UV chromatogram is shown at the top and a RP-HPLC-FL chromatogram is shown at the bottom. The chromatograms are stacked and both the ordinate and abscissa axes are aligned. Effluent from a reversed-phase column was fed to the Model 2478 UV absorption detector and the effluent from this detector was made the influent to the Model 474 fluorescence detector as shown in

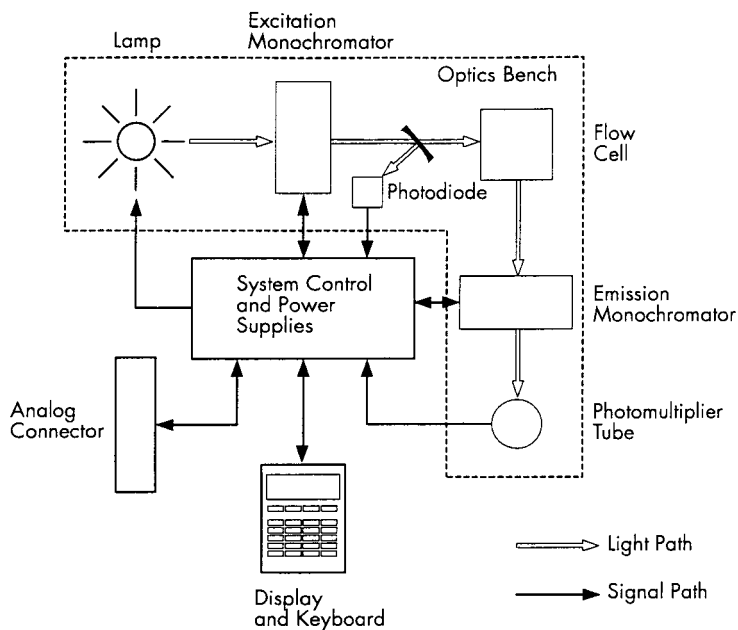


FIGURE 4.55 Schematic of a fluorescence HPLC detector showing microprocessor control and analog output.

Fig. 4.57. Analog signals from both detectors are sent through an A/D converter to a central PC workstation that uses Turbochrom (P-E Nelson) to acquire the data and to generate the chromatograms. The peaks in both chromatograms of Fig. 4.56 are due to the injection of a multicomponent standard of the 16 priority pollutant PAHs. They elute in the order shown in Table 4.17.

We have completed our discussion of the determinative techniques for the quantitative determination of organic compounds that are of concern to TEQA. What's left? Well, let us return to Fig. 4.1 and consider that region in the chromatography world that deals with the analysis of ionic substances. We want to be able to identify ionically bonded chemical substances that are of concern to TEQA and to consider what determinative technique one would find in trace environmental testing labs today. We must then discuss how ion chromatography (IC) became the dominant determinative technique to separate, identify, and detect trace concentrations of inorganic ions in aqueous samples of environmental interest.

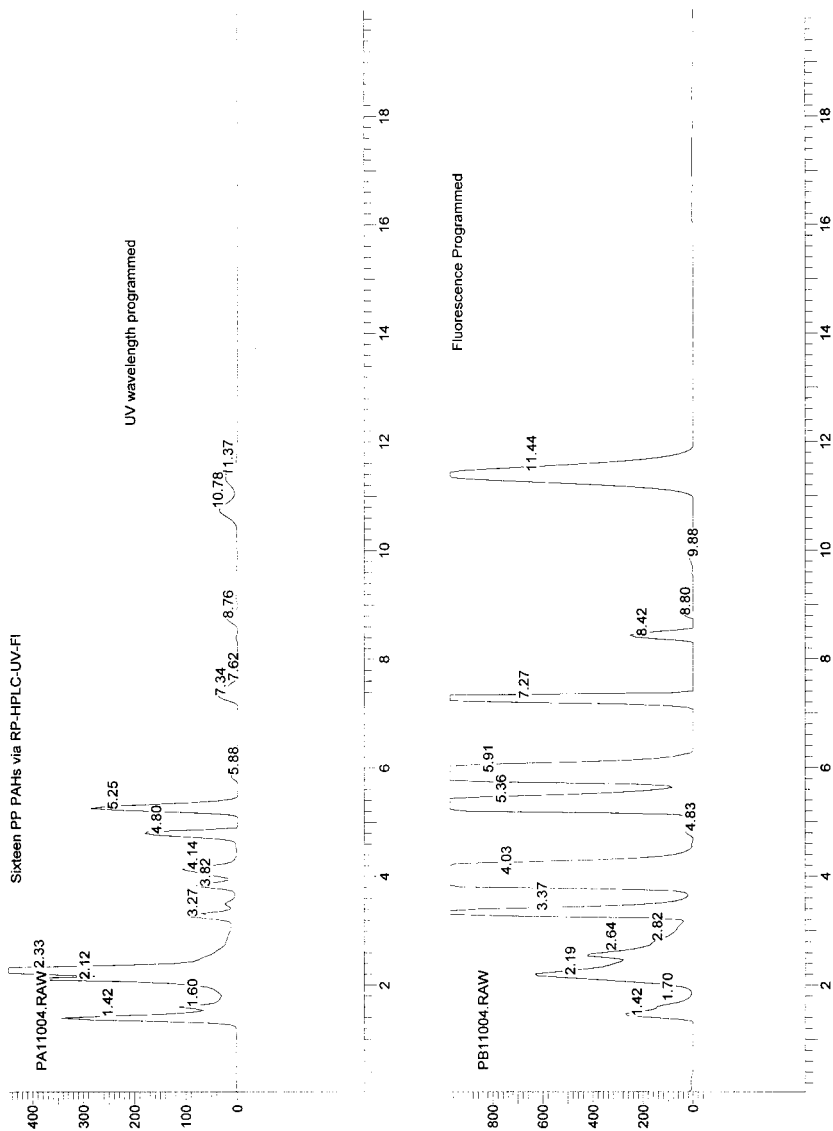


FIGURE 4.56 Comparison of RP-HPLC chromatograms for the separation and detection of a 1-ppm reference standard containing the 16 priority pollutant PAHs between an UV absorbance and fluorescence HPLC detector.

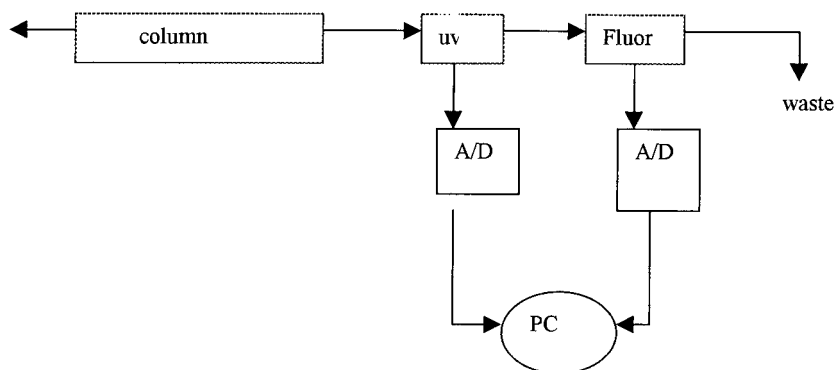


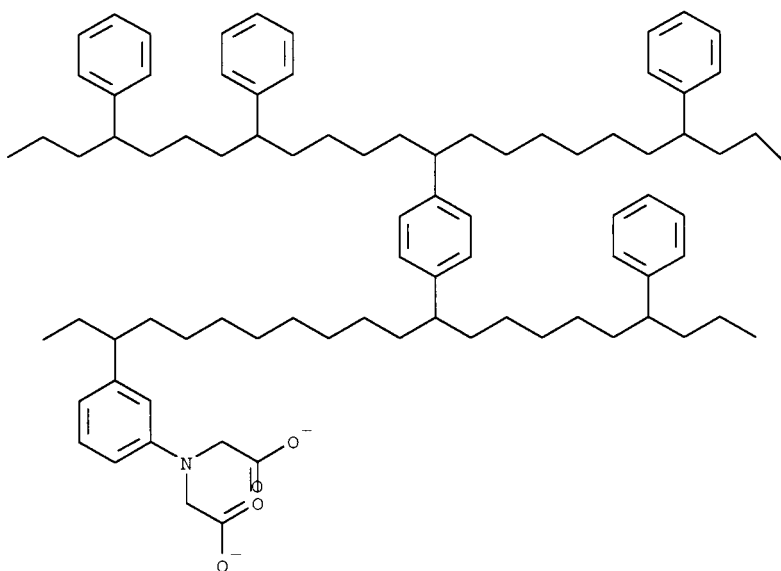
FIGURE 4.57 Schematic for the configuration in the author's lab of the single HPLC column and dual UV absorbance and fluorescence detectors placed in series showing the relationship to a PC.

TABLE 4.17 Summary of the Number of Fused Rings Associated with the Priority Pollutant PAHs

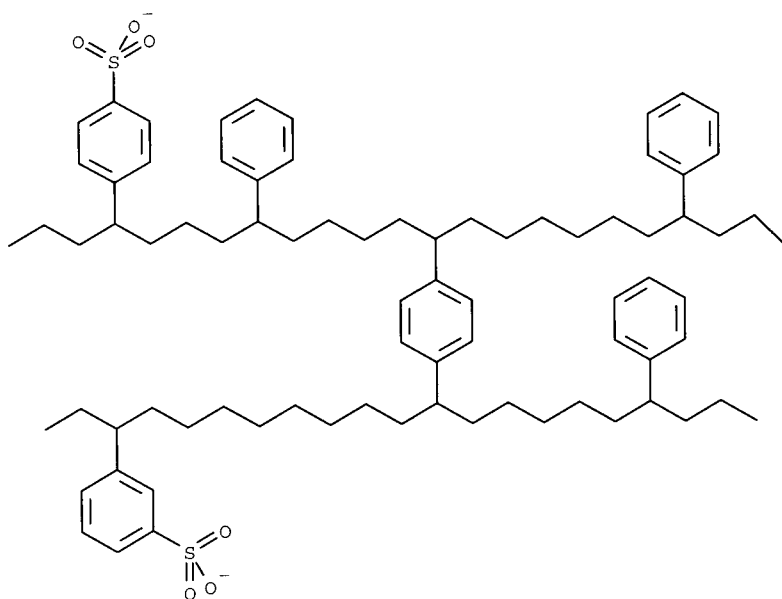
No.	No. of rings	Name
1	2	Naphthalene
2	2	Acenaphthylene
3	2	Fluorene
4	2	Acenaphthene
5	3	Phenanthracene
6	3	Anthracene
7	3	Fluoranthene
8	4	Pyrene
9	4	Benzo[<i>a</i>]anthracene
10	4	Chrysene
11	5	Benzo[<i>b</i>]fluoranthene
12	5	Benzo[<i>k</i>]fluoranthene
13	5	Benzo[<i>a</i>]pyrene
14	5	Dibenzo[<i>a, h</i>]anthracene
15	6	Indeno[1,2,3- <i>cd</i>]pyrene
16		Benzo[<i>ghi</i>]perylene

60. HOW DID IC MAKE IT TO THE FOREFRONT IN TRACE INORGANICS ANALYSIS?

To answer in brief, by evolving from a low-pressure large-column-diameter (> 1 cm) ion-exchange chromatography (IEC) technique to a high-pressure, narrow-column-diameter (~ 5 mm) high-performance IEC technique! The use of glass columns packed with either a strong or weak anion- or cation-exchange resin is routine in many chemical laboratories. However, the development of IC as an additional determinative technique very useful to TEQA requires that we discuss these principles. But first, as a way to review some principles of conventional IEC, let us discuss an ingenious use of two chemically different ion-exchange resins and the simple experiment that can illustrate very nicely the principle of ion-exchange (IE). One of the most useful IE resins is Chelex-100, 1% cross-linked, 50–100 mesh, Na^+ form (Sigma). Chelex-100 is a styrene–divinylbenzene resin containing iminodiacetate groups. Its structure is shown as follows:



Conventional cation-exchange resin such as AG 50W-X8, 8% cross-linked, 20–50 mesh, H^+ form (Bio-Rad), whose IE capacity is 5.1 mEq/g is a sulfonic acid type. The structure of this resin is:



Chelex-100 in its Na^+ form can exchange other metal ions such as Fe^{2+} , whereas AG 50W-X8, in the H^+ form exchanges other metal ions such as Na^+ for H^+ . The author recently packed two conventional glass columns, one containing Chelex-100 and the other containing AG 50W-X8, as shown in Fig. 4.58. Suppose that we pass an aqueous solution containing Fe^{2+} through the first column in Fig. 4.58. If we then take the effluent from the first column and place it on top of the second column, we discover, after enough aqueous solution has been passed through this column, that the effluent is quite acidic (i.e., its pH is < 4). This solution can be titrated against a standardized base such as $0.02M$ NaOH to a bromocresol end point and from the number of milliliters of titrant required, the number of millimoles of H^+ can be obtained. This many millimoles is also the number of millimoles of Na^+ that exchanged for H^+ . For every millimole of Fe^{2+} exchanged on the first column, 2 millimoles of Na^+ ions must have been released. Hence, the number of mL of $0.02M$ NaOH can be indirectly related to the number of millimoles of iron(II) present in the original sample! The stoichiometry for this series of chemical reactions is as follows:

Chemical Reactions Used in This Demonstration

Column to the left:



Column to the right:



At the buret:



Bromocresol-Green turns *yellow* when the pH is below 4.0 and *blue* when the pH is above 5.6

The appearance of a gradual darkening of the head of the packed column that contains Chelex-100 is observed at first. This amount of darkening of the white resin gets larger and larger as successive aliquots of the iron(II) solution are added on top of the column. The brownish AG 50W-X8 does not change color during these repetitive additions of sodium ion to the top of this column. Let us return to our development of the principles of IC.

Two achievements that led to the acceptance of IC as an important determinative technique in TEQA are now discussed. The first was the development of surface-agglomerated low-capacity anion- and cation-exchange resins with particle sizes appropriate for preparing high-performance IC columns. The second was the realization that to detect trace concentration levels in a background of highly conducting IC eluent required a “chemical suppression” of the eluent conductivity. The research group led by Small at Dow Chemical in the early 1970s is credited with the pioneer development of suppressed IC (96) and a portion of their abstract to this benchmark paper follows:

Ion exchange resins have a well known ability to provide excellent separation of ions, but the automated analysis of the eluted species is often frustrated by the presence of the background electrolyte used for elution. By using a novel combination of resins, we have succeeded in neutralizing or suppressing this background without significantly affecting the species being analyzed which in turns permit the use of a conductivity cell as a universal and very sensitive monitor of all ionic species either cationic or anionic.

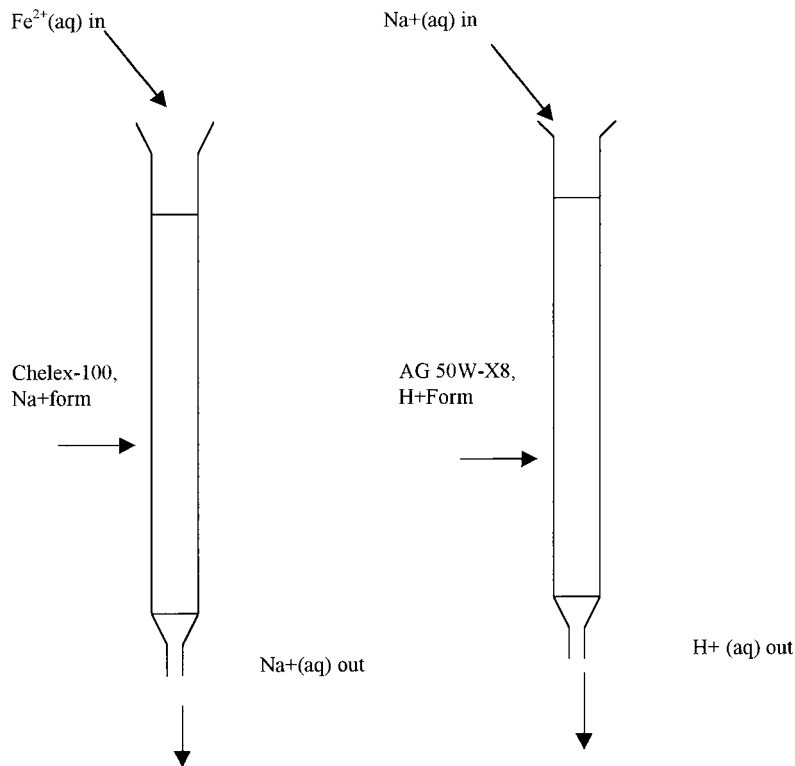


FIGURE 4.58 Schematic of two gravity-fed low-pressure LC columns packed with ion-exchange resins.

This benchmark publication was followed by a series of developments that continues to this day in the evolution of IC as a trace determinative technique. The Dionex Corporation was formed out of these developments from Dow Chemical in the mid-1970s and this company has been quite an innovator in the manufacture of instrumentation for suppressed IC. From the earlier model such as the 10 to the 2000I to the DX 500; from the cation-exchange column to the hollow-fiber cation suppressor to the micro-membrane cation suppressor to the self-regenerate cation suppression for anion analysis and now to their “just add water” slogan, Dionex Corporation has made considerable advances in IC technology. A nonsuppressed form of IC also developed during the 1970s. To quote from the author’s abstract of this benchmark paper (97):

The anions are separated on a column containing a macroporous anion-exchange resin which has a very low exchange capacity of

0.007 – 0.07 milli-equivalents per gram. Because of the low resin capacity, only a very dilute solution, $1 \times 10^{-4}M$ of an aromatic organic acid salt is needed as the eluent. The eluent conductance is sufficiently low that a suppressor column is not needed, and the separated anions can be detected with a simple conductance detector.

Unfortunately, nonsuppressed IC has not been developed commercially and remains an interesting determinative technique for academic labs. It took, however, over 10 years for the EPA to approve of suppressed IC to quantitatively determine the common inorganic anions derived from moderate to strong acids such as fluoride, chloride, nitrite, nitrate, phosphate, and sulfate in drinking water! Years of effort have resulted in EPA Method 300.1 “The Determination of Inorganic Anions in Drinking Water by Ion Chromatography.” This method considers two different sets of inorganic anions in drinking water, reagent, surface water, and groundwater. The first set are the classical ones listed earlier and the second set consists of analytes derived from the so-called disinfection by-products (DBPs). This second set includes bromate, bromide, chlorite, and chlorate (oxyhalides). The method is replete with sufficient quality control, something that was seriously lacking in the earlier versions of Method 300.

61. HOW DOES AN IC WORK?

An ion chromatograph using a suppressed IC mode of operation can be viewed as a moderate-pressure-performance liquid chromatograph. A schematic of the essential components of an IC is shown in Fig. 4.59. Because the trace concentration levels of various inorganic anions is of most interest to TEQA, we will focus our discussions on how anions are measured. Alkali and alkaline-earth metal ions and the ammonium ion are common applications of IC. In fact, NH_4^+ can only be measured chromatographically by cation IC! The transition metal ions can also be separated and detected, however, atomic spectroscopy, to be discussed after we complete IC, is the predominant TEQA determinative technique. We will not discuss the separation and detection of cations here.

Referring to Fig. 4.59 and focusing on trace anion analysis, the simplest ion chromatograph can be viewed as consisting of four modes: (a) a delivery mode that using a bicarbonate/carbonate buffer as the mobile-phase eluent and a single-piston reciprocating pump with check valves and pressure gauge/dampener accessory and a means to introduce a fixed volume of sample; (b) a separation mode that consists of a column packed with a low-capacity anion-exchange resin; (c) a detection mode that includes

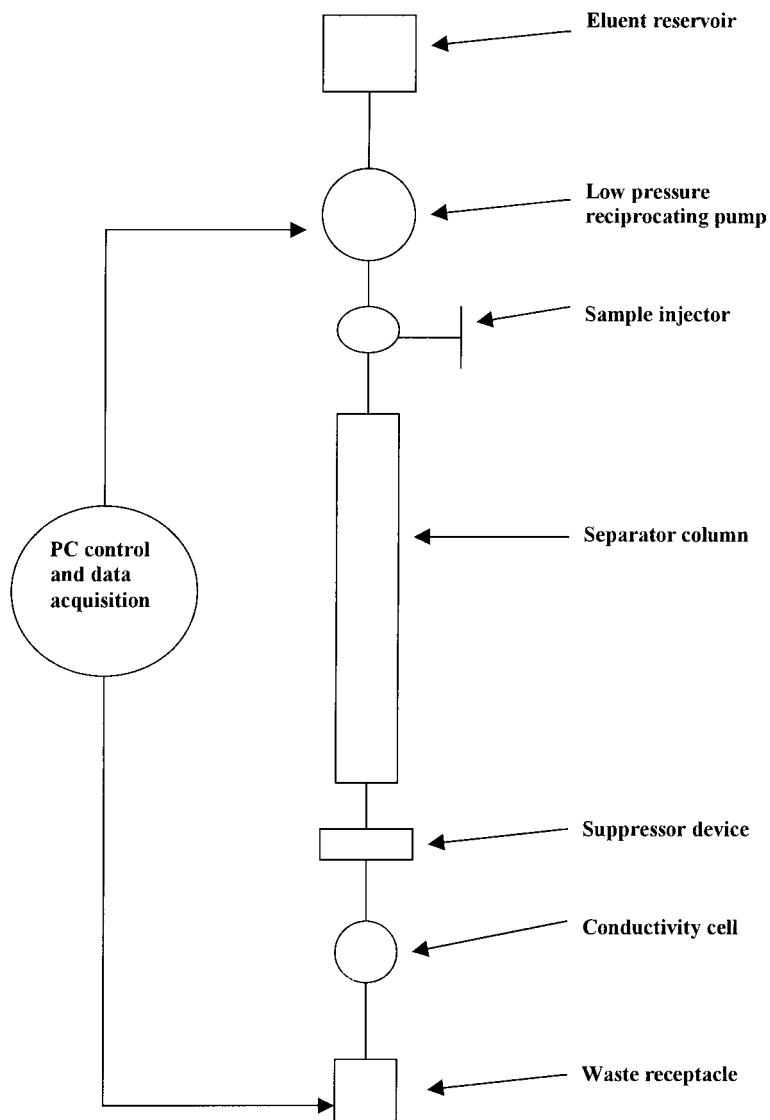


FIGURE 4.59 Schematic diagram for a generalized view of a typical suppressed ion chromatograph.

a suppressor device and a micro-flow conductivity cell; (d) a data mode that could be either a strip-chart recorder, an electronic integrator (one made by either Spectra-Physics or Hewlett-Packard were popular at one time), or a PC via a A/D converter. In the author's laboratory, the analog signal from the conductivity cell is connected to a 900 interface box (P-E Nelson). The interface is connected to the input of a PC using a IEEE cable. The PC utilizes Turbochrom (P-E Nelson) chromatography data acquisition software in a Windows[®] disk operating environment.

It was critical to the development of IC to achieve the IE separation of the common inorganic anions in a reasonable length of time (e.g., within 20 min until the SO_4^{2-} elutes) and with sufficient chromatographic resolution. It appeared inevitable that advances in HPLC made in the early 1970s would simultaneously advance the chromatographic separation of ions or ionizable compounds. The drastic lowering of particle size, predicted by the contributions to plate height and the realization that pellicular materials minimize the stationary-phase thickness lead to a more chromatographically efficient IC column. Ways to lower the IE capacity were pursued and these efforts resulted in more efficient columns and significantly lower values for k' .

To achieve this increase in IC column efficiency, IE resins had to be developed that differed significantly from those of conventional resins. Table 4.18 taken from an earlier article compares the properties of conventional IE resins to those required for IC (98). A decrease in IE capacity by about two orders of magnitude permitted the use of eluents for IC whose concentrations are three orders of magnitude lower than those used in conventional IE analysis. Eluent concentrations in IC are routinely prepared at the millimolar level. The favorable IE properties were realized with the development of surface-agglomerated pellicular resins. Such a pellicular

TABLE 4.18 Comparison of Conventional to Surface-Agglomerated Ion-Exchange Resin

Conventional resin	Surface-agglomerated resin
Capacity: 3–5 mEq/g	Capacity: 0.02 mEq/g
Large degree of swelling	Very little swelling
Many diffusion paths	Limited diffusion paths
Separation by ion size	Little separation by ion size
Not easily poisoned	Easily poisoned but can be regenerated
High sample concentrations	Low sample concentrations
High eluent strength	Low eluent strength

Source: Ref. 98.

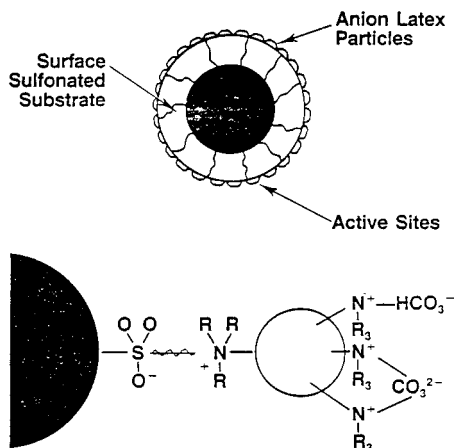


FIGURE 4.60 Surface-agglomerated pellicular particles used in low-capacity anion-exchange columns for IC.

particle is shown in Fig. 4.60. A surface-sulfonated particle is shown agglomerated with an anion latex particle. The illustration shows the bicarbonate and carbonate ions, the most common eluent ions used in anion IC, as attached to the latex particle. The chemistry involved in separating chloride from sulfate and being able to quantitate these common ions found in groundwaters is shown schematically in Fig. 4.61. The eluent used in this schematic is NaOH so that the counterion is hydroxide ion as shown as the analyte ion symbolized as X^- is chromatographed through the separator column. As the eluent and analyte ions reach the suppressor, prior to the conductivity detector, as shown in Fig. 4.61, the hydroxide ion eluent is converted to the minimally conducting water and the analyte ion is converted to the highly conducting strong acids HCl and H_2SO_4 . It is well known that the H^+ and the OH^- are significantly more conductive than are any of the common anions.

62. IS THERE A THEORETICAL BASIS FOR CONDUCTIVITY MEASUREMENTS?

Let us digress a moment and discuss the relationships involving conductivity (99). An aqueous solution containing dissolved inorganic salts or electrolytes conducts electric current. The extent of this conductance, denoted by G , is defined in terms of the electrical resistance of the electrolytic solution. The reciprocal of this resistance is the conductance such that $G = 1/R$ and

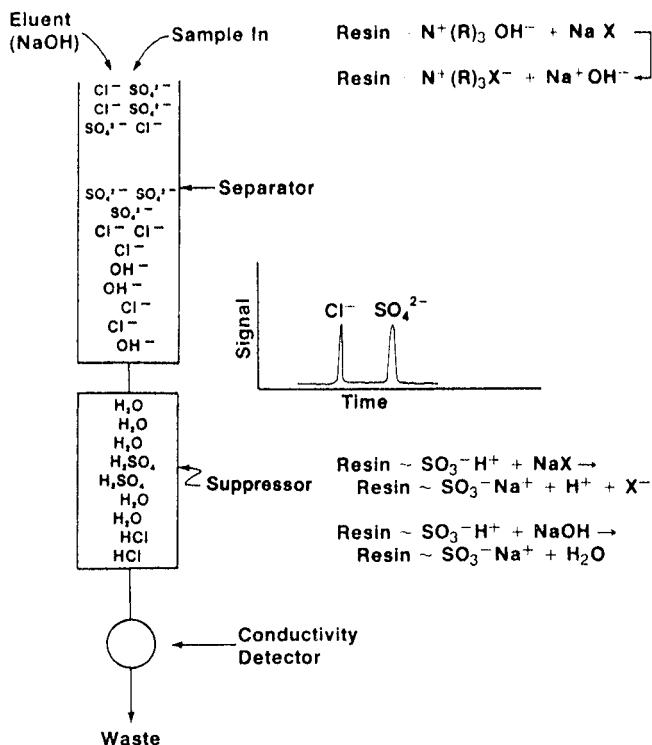


FIGURE 4.61 Schematic of IC showing the ion-exchange chemistry.

the conductance is expressed in units of Ω^{-1} or siemen, denoted by S. An older unit is the mho which is ohm spelled backward. Conductance is a bulk property and encompasses both the solute and solvent that pass through a conductivity detector. A solution has a specific conductivity κ in units of siemens per centimeter for a typical conductivity cell. This cell consists of a pair of identical electrodes each having a surface area A and spaced 1 cm apart. An AC voltage is applied across the electrodes and the resistance measured. A cell constant k can be defined as the ratio of the spacing distance to surface area (l/A) and all of this gives

$$\kappa = \frac{Gl}{A} = Gk$$

Conductivity as a analyte-specific property can be considered by first defining the equivalent limiting conductivity, denoted by Λ , for a typical inor-

ganic salt, MX_n where M^{n+} is a cation of positive charge n and X is a monatomic anion (the selection of a monatomic anion serves to simplify the equations). Λ is the conductance if enough of the salt was available to equal 1 gram-equivalent weight in a liter of solution. Hypothetically, this would require very large electrode surface areas while maintaining the same spacing between electrodes at 1 cm! This is summarized in the following relationship:

$$\Lambda = \frac{1000k}{C}$$

For a concentration C of the salt MX_n dissolved in water (in equivalents per liter), Λ has units of siemens per square centimeter per equivalent. By substitution and rearranging, the conductance G can be related to the equivalent limiting conductivity and Λ . The use of Λ enables one to consider the separate contributions of cation and anion. The conductance can then be related to the concentration of the salt, the separate limiting conductances of the individual ions, and the cell constant k as follows:

$$G = \frac{C\Lambda}{1000k} = \frac{C_{\text{MX}}}{1000k}(\lambda_{\text{M}} + \lambda_{\text{X}})$$

We see from the equation that each cation, M^{n+} , and each anion, X^- , can be given its own value for λ .

We now proceed to relate the bulk property G to the analyte concentration and thus connect instrumental IC to TEQA. We focus only on anion-exchange IC; however, these relationships can also be derived from the perspective of the cation using cation-exchange principles. We consider any monatomic anion X , where X could be chloride, nitrate, and so forth, in a background of eluent such as E^+E^- , where E^+ might be Na^+ and E^- might be CO_3^{2-} . Let us assume that the concentration of anion is C_{X} and the degree of ionization of this anion is α_{X} . Because the anion displaces the anions in the eluent as it passes from the column to the detector, we can state that the concentration of eluent is

$$C_{\text{E}} - C_{\text{X}}\alpha_{\text{X}}$$

Let us consider the nature of the conductance G during chromatographic elution of the salt MX_n such that the following relationship holds:

$$G_{\text{total}} = G_{\text{eluent}} + G_{\text{anion}}$$

Upon substituting the equation that relates limiting equivalent conductances for separate ions to the conductance of a solution, we have

$$G_{\text{total}} = \frac{(\lambda_{\text{E}^+} + \lambda_{\text{E}^-})(C_{\text{E}} - C_{\text{X}}\alpha_{\text{X}})\alpha_{\text{E}}}{1000k} + \frac{(\lambda_{\text{E}^+} + \lambda_{\text{X}^-})C_{\text{X}}\alpha_{\text{X}}}{1000k}$$

At the moment the anion is detected, there is a change in the total conductance such that

$$\Delta G = G_{\text{total}} - G_{\text{backgd}}$$

The background conductance can be expressed in terms of the eluent cation and anion, respectively and, when subtracted from the total conductance, leads to an important relationship according to

$$\Delta G = \left[\frac{(\lambda_{\text{E}^+} + \lambda_{\text{X}^-})\alpha_{\text{X}} - (\lambda_{\text{E}^+} + \lambda_{\text{E}^-})\alpha_{\text{E}}\alpha_{\text{X}}}{1000k} \right] C_{\text{X}}$$

This equation is the most general of all and accounts for both suppressed and nonsuppressed IC. Note that the degree of eluent ionization, α_{E} , has a significant effect on the magnitude of ΔG . In the case of suppressed IC, the eluent cation, E^+E^- is converted to H^+E^- while the analyte salt MX_n is converted to highly conductive H^+X^- . The creative idea here was to recognize that if E^+E^- could be made from a moderate to strong base, such as NaOH , Na_2CO_3 , or NaHCO_3 , then H^+E^- that is formed in the suppressor is rendered minimally conductive!

If the eluent and anions are fully ionized, the above equation can be further simplified to give us a more direct relationship between the change in conductance and anion concentration:

$$\Delta G = \frac{(\lambda_{\text{A}^-} - \lambda_{\text{E}^-})C_{\text{X}}}{1000k}$$

We thus see that the magnitude of the change in conductance depends not only on anion concentration but also on the difference between the limiting equivalent conductances of the anion and the eluent ion (99).

The idea behind eluent suppression has been to convert the moderately conductive Na^+ in the eluent to the weakly conductive H_2CO_3 while converting the low-conducting analyte NaX to the high-conducting HX . Limiting equivalent conductivity, denoted by λ , of some ions in aqueous solution at 25°C are as follows (100):

Anion/cation	λ (mho-cm ² /Eq)
H ⁺	350
OH ⁻	198
SO ₄ ²⁻	80
Cl ⁻	76
K ⁺	74
NH ₄ ⁺	73
Pb ²⁺	71

It is this author's opinion that the development of the micro-membrane suppressor was the key advance that stabilized the background conductivity. A schematic of this suppressor is shown in Fig. 4.62. The suppressor can be viewed as a "sandwich" whereby the "bread" represents eluent and regenerant flow in opposite directions and the "meat" is a semipermeable cation-exchange membrane. This membrane allows H⁺ to pass through and neutralize the carbonate eluent by converting it to the weakly conductive H₂CO₃. Figure 4.63 shows several ion chromatograms obtained by the author during the late 1980s. The top IC chromatogram shows the separation and detection of all seven of the common inorganic anions in less than 7 min using a AS-4 anion-exchange column (Dionex) with an eluent consisting of 17 mM NaHCO₃/18 mM Na₂CO₃ with a flow rate of 2.0 mL/min. Referring back to Fig. 4.59, this Model 2000I (Dionex) was connected to an electronic integrator at the time to generate the printout shown in Fig. 4.63. The bottom IC chromatogram is that of a typical groundwater sample which identifies chloride and sulfate. These ions are very common in groundwater, revealing an unidentified peak at

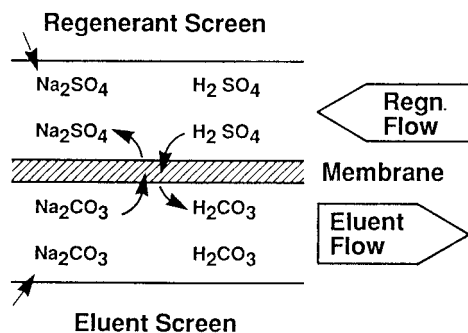


FIGURE 4.62 Schematic of the micro-membrane suppressor used in IC.

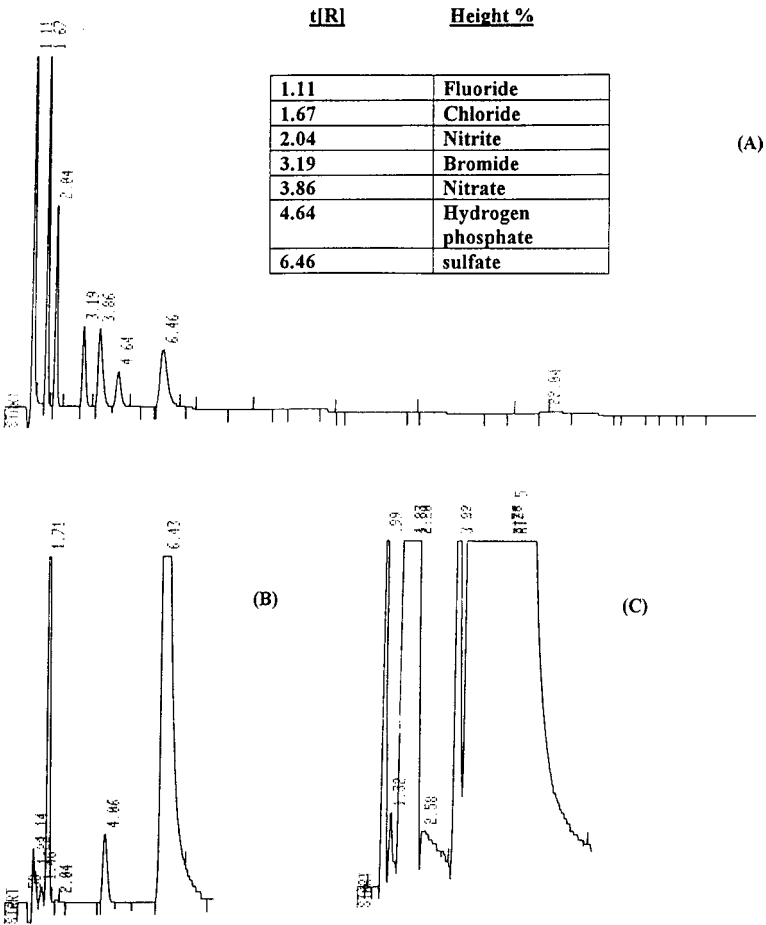


FIGURE 4.63 Actual IC readouts from an integrator obtained by the author. IC conditions; column: AS-4®(Dionex); flow rate: 2 mL/min; eluent: 0.0017M HCO₃⁻/0.0018M CO₃²⁻; attenuation @ 6; sensitivity: 300 μS full scale; sample: A) 6ppm inorganic anions standard, B) typical groundwater sample showing significant concentraton levels for chloride and sulfate, and C) groundwater sample "fixed" with sulfuric acid.

a retention time of 4.06 min. In the chromatogram adjacent to this one, a second groundwater sample that happened to be preserved with sulfuric acid is shown. This IC chromatogram indicates that preserving aqueous samples from the environment for subsequent analysis by IC with H₂SO₄ is a real mistake!

63. IF IC IS NOT THE PRINCIPAL DETERMINATIVE TECHNIQUE TO MEASURE METALS IN THE ENVIRONMENT, WHAT IS?

Atomic emission spectroscopy (AES) and atomic absorption spectroscopy (AAS) are! In a manner similar to our discussion of molecular spectroscopy, where we compared UV absorption with UV excitation and subsequent fluorescence, these two determinative approaches are the principal ways to identify and quantitate trace concentration levels of metal contamination in the environment. As the need developed to quantitate increasing numbers of chemical elements in the Periodic Table, so too came advances in instrumentation that enabled this to be achieved at lower and lower IDLs! AES and AAS techniques are both complementary and competitive. Atomic fluorescence spectroscopy (AFS) is a third approach to trace metal analysis. However, instrumentation for this has not as yet become widespread in environmental testing labs and it is unlikely that one would see atomic or what has become useful x-ray atomic fluorescence spectroscopy. Outside of a brief mention of the configuration for AFS, we will not cover it here.

We begin this section of Chapter 4 with a discussion of spectroscopy and it is useful for us to briefly survey all of molecular and atomic spectroscopy from the perspective of the electromagnetic spectrum. The entire electromagnetic spectrum is shown in Fig. 4.64, along with those regions in the spectrum that are associated with different types of radiation. The various regions of the electromagnetic spectrum and their respective interactions with matter are cited below each region. The wavelength region starting from the near-UV (~ 200 nm) through to the near-infrared (~ 800 nm) is used in atomic spectroscopy and it involves transitions of electrons between ground and excited electronic states. Spectroscopy, in general, has been defined as that branch of physical chemistry that considers the interaction of electromagnetic radiation with matter. Harris has described what it is that atomic spectroscopy does (101):

In atomic spectroscopy, samples are vaporized at very high temperatures and the concentrations of selected atoms are determined by measuring absorption and emission at their characteristic wavelengths. Because of its high sensitivity and the ease with which many samples can be examined, atomic spectroscopy has become one of the principal tools of analytical chemistry.

The quantitative determination of trace concentration levels of the priority pollutant metals is a major activity in environmental testing laboratories. Instruments are designed to accept aqueous samples directly and convert these samples to aerosols or fine mists that are aspirated into

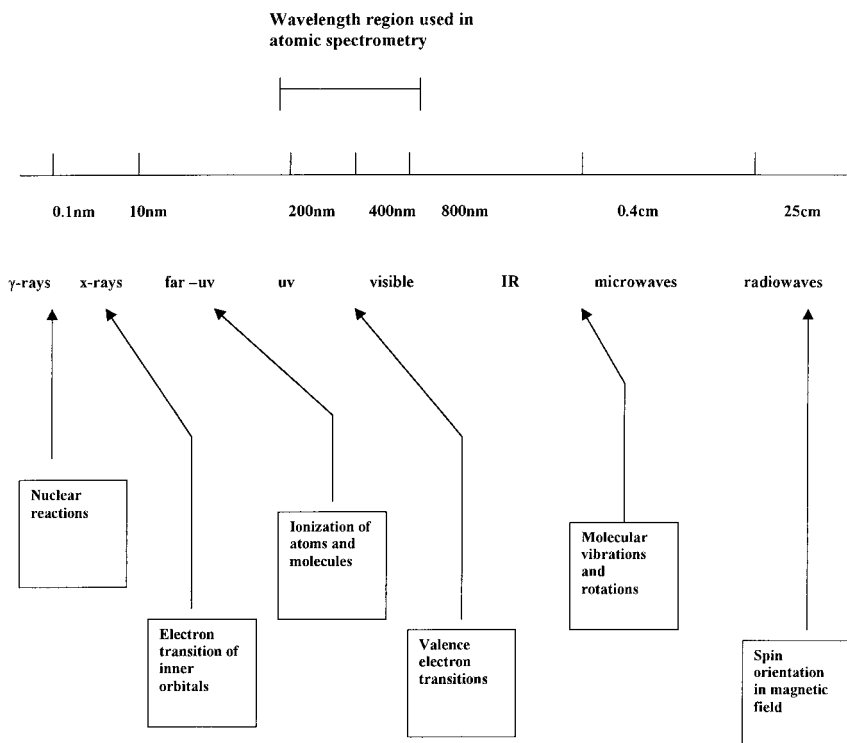


FIGURE 4.64 The electromagnetic spectrum and its relationship to nuclear, atomic, and molecular spectroscopy.

flames or plasmas. Solids must be solubilized by vigorous use of strong acids and hydrogen peroxide. This oxidation results in an acidic/aqueous sample that can be subsequently aspirated into a flame or plasma or placed in a resistively heated graphite tube. A logic flowchart is shown in Fig. 4.65 and the decision as to which determinative instrumental technique to use largely depends on the required IDLs, or in more familiar terms, “how low can you go!” The logic presented in Fig. 4.65 should enable the reader to make the right decision given the available instrumentation found in a typical environmental testing laboratory. If reaching the IDLs as indicated in Fig. 4.65 is not necessary, there exists a plethora of analytical methods for metal ions in aqueous solution based on metal chelation with subsequent quantitative analysis using a stand-alone UV-vis spectrophotometer. As introduced in Chapter 3, LLE or alternative column chromatographic techniques for sample prep are available to iso-

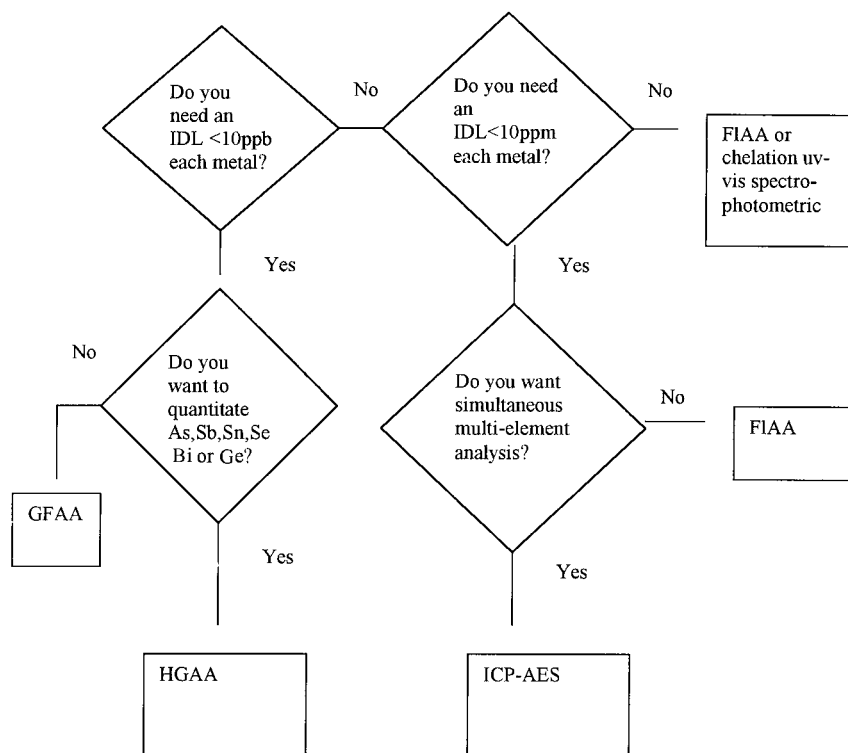


FIGURE 4.65 Logical flowchart for choosing which atomic spectrometric determinative technique to use.

late and/or preconcentrate metal chelates from complex sample matrices. The reference work by Sandell and Onishi on the UV-vis spectrophotometry of metals is the most complete work of which this author is aware (102). One experiment in Chapter 5 shows how one can quantitate the two oxidation states of the element iron, namely Fe(II) and Fe(III), by forming a metal chelate and using a stand-alone UV-vis spectrophotometer.

64. HOW DO I CHOOSE WHICH ATOMIC SPECTRAL METHOD TO USE?

Most alkali and alkaline-earth metal ions that are found dissolved in water are readily quantitated by flame atomic emission spectroscopy (FI-AES). This determinative technique has been, in the past, termed "flame photometry." A simple photometer uses cutoff filters to isolate the wavelength,

denoted by λ , with the strongest emission intensity that usually originates or terminates from a resonance line. Resonance transitions are those originating from or terminating to the ground electronic state. For example, the intensity of the 589/590-nm resonance atomic emission doublet for sodium is used to quantitate Na in aqueous samples. Contemporary atomic absorption spectrophotometers incorporate a monochromator and are used in the FI-AES mode by merely not using the hollow-cathode lamp. The transition or so-called “heavy” metal ions dissolved in water are more amenable to quantitative analysis using flame atomic absorption (FIAA), graphite furnace atomic absorption (GFAA), inductively coupled plasma-atomic emission (ICP-AES), or ICP integrated to a quadrupole mass filter. This relatively recent development is termed ICP-MS. Table 4.19 lists current IDLs for selective metals of interest to TEQA (103). The IDLs cited make it quite clear as to which atomic spectroscopic determinative technique is more appropriate.

The following example illustrates how one can decide among these several options. Consider a request from an environmental engineering firm involving the evaluation of a landfill that is suspected to contain the toxic element arsenic (As). This element is part metal and part nonmetal owing to its position in the Periodic Table. Atoms of the element As are usually found covalently bonded to oxygen either in a trivalent, denoted by As(III), or in a pentavalent, denoted by As(V), oxidation state. Arsenic speciation (i.e., the quantitative analysis of environmental samples) for both As(III) and As(V) is an important and current area of research interest. We will have more to say about metal speciation and its growing importance to TEQA later in this chapter. AES and AAS determinative techniques yield total As without regard to speciation. As is difficult to measure down to low IDLs by FIAA. The drinking water regulatory limit for As is set at 50 ppb As. The IDLs listed in Table 14.9 for As suggest that of the several determinative techniques available, either GFAA with an IDL of 0.5ppb, HGAA with an IDL of 0.03 ppb or ICP-MS with an IDL of 0.006ppb, if available, is the recommended determinative technique. Recently, considerations such as these for As have become critical in the author’s laboratory. The decision as to which atomic spectroscopic instrument to choose in TEQA as outlined in Fig. 4.65 is analogous to the choice as to which type of instrumental chromatographic separation determinative technique to use, as outlined in Fig. 4.1. We proceed now to discuss the principles underlying AES, then we will introduce AAS, and, finally, we discuss determinative techniques to address the problem of metal speciation in TEQA. But first, how did this all begin?

TABLE 4.19 IDLs for the Most Common Atomic Spectrometric Determinative Techniques in ppb

Element	FIAA	GFAA	HGAA	ICP-AES	ICP-MS
Al	45	0.3		6	0.006
As	150	0.5	0.03	30	0.006
Bi	30	0.6	0.03	30	0.0005
Ca	1.5	0.03		0.15	2
Cd	0.8	0.02		1.5	0.003
Cr	3	0.08		3	0.02
Cu	1.5	0.25		1.5	0.003
Fe	5	0.3		1.5	0.04
Hg	300	15	0.009	30	0.004
K	3	0.02		75	1
La	3000			1.5	0.0005
Mg	0.15	0.01		0.15	0.007
Na	0.3	0.05		6	0.05
Pb	15	0.15		30	0.001
Sb	45	0.4	0.15	90	0.001
Se	100	0.7	0.03	90	0.06
Sn	150	0.5		60	0.002
Tl	15	0.4		60	0.0005
Zn	1.5	0.3		1.5	0.003

Note: Instruments used to generate these IDLs:

- Model 5100 AA (Perkin-Elmer, P-E)
- FIAS-200 flow injection amalgamation (P-E)
- MHS-10 mercury/hydride (P-E)
- Model 5100 PC AA with 5100 ZL Zeeman furnace model (P-E)
- Model 4100 ZL AA
- Plasma 2000 ICP-AES (P-E)
- ELAN 5000 ICP-MS (P-E)

All IDLs are based on a 98% (3σ) confidence level.

Source: Ref. 103.

65. WHAT HAPPENS WHEN VARIOUS INORGANIC SALTS ARE THRUST INTO FLAMES?

We start with the observations of Kirchhoff and Bunsen in Germany in 1859. They observed the “bright line” spectra for many alkali and alkaline-earth metal-based salts and are credited with the discovery of spectro-chemical analysis. The so-called principal atomic emission series for the common alkali metals is shown in Fig. 4.66. Note that these older atomic spectra are calibrated in terms of wave number, denoted by $\bar{\nu}$, of the emitted radiation whose units are in reciprocal centimeters (denoted by cm^{-1}).

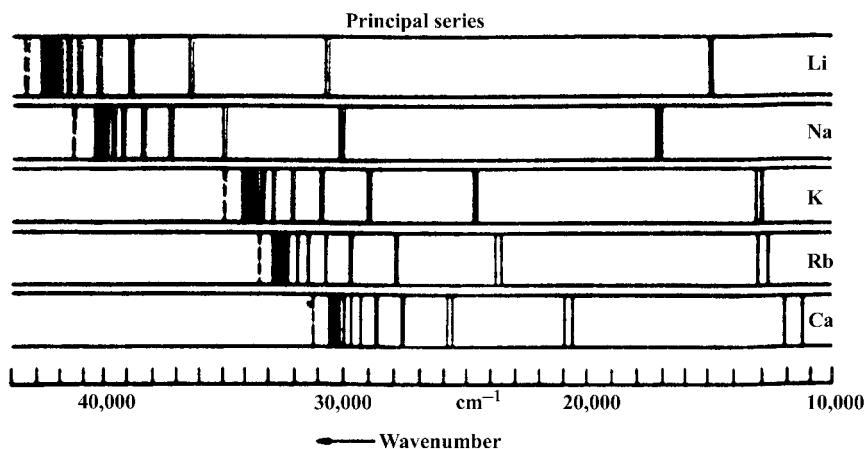


FIGURE 4.66 Principal atomic emission lines for the alkali metals.

Contemporary discussions express the wavelength in units of nanometers (denoted by nm). Frequency is expressed in units of cycles per second or Hertz (denoted by Hz). Frequency can also be viewed in terms of the number of wavelengths per second as $\nu = c/\lambda$. Can we convert between the two? From Planck's law, we know that the energy carried by these emitted photons is inversely related to the frequency λ , directly related to ν , and directly related to $\bar{\nu}$, and this is summarized as

$$E = h\nu = hc\bar{\nu}$$

A rule of thumb between wavelength (in nm) and wave number (cm^{-1}) is given by

$$\lambda (\text{nm}) = \frac{1 \times 10^7}{\bar{\nu} (\text{cm}^{-1})}$$

We introduce the term *wave number* at this point because it relates directly to energy and wave number is the principal unit used for the mid-infrared region of the electromagnetic spectrum. In Fig. 4.66, the Na doublet resonance emission line occurs at $\bar{\nu} \sim 17,000 \text{ cm}^{-1}$. Applying the above equation gives $\nu = 588 \text{ nm}$ and this value is quite close to the 589/590-nm doublet.

The alkali metals all have a lone electron in an outermost *s* atomic orbital. The transition from the higher *p* atomic orbital in the excited state to this *s* orbital corresponds to an energy difference ΔE that can be attributed to release of a photon whose energy is $h\nu$. Quantum mechanical selection rules

allow a change in the azimuthal quantum number $\Delta l = +1$ or $\Delta l = -1$. Each transition gives rise to a doublet because the spin $s = \frac{1}{2}$ may couple either as $l + s = \frac{3}{2}$ or $l - s = \frac{1}{2}$ in the p state. The separation of the doublet of Na and K has been exaggerated in Fig. 4.66. One of the more fascinating observations in the introductory general chemistry laboratory is to observe the various colors emitted when the salts NaCl, LiCl, KCl, CaCl₂, and SrCl₂ are thrust into a lab burner flame. Bright line spectra result when this luminescence is directed through a prism or transmission grating such that the bright “light” is dispersed into its component λ 's.

A partial energy-level diagram is shown in Fig. 4.67 for the element Na. Note the two closely spaced levels for $3p$ electrons that account for the most intense 589/590-nm doublet. AE results from a relaxation of the elec-

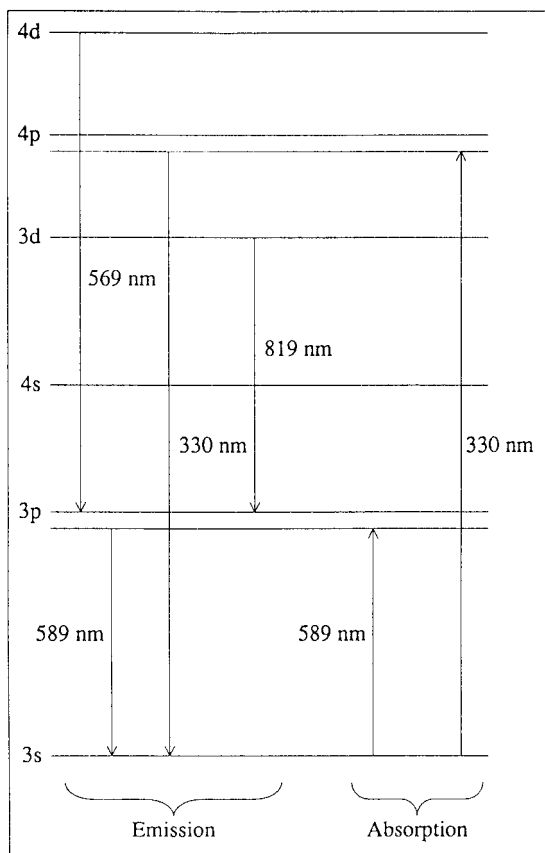


FIGURE 4.67 Partial energy-level diagram for sodium.

tron from the excited $3p$ atomic orbital to the ground-state s atomic orbital and this process is depicted as



The energy emitted as photons according to Planck's law is depicted as

$$E_{3p} - E_{3s} = \Delta E = h\nu$$

Provided sodium vapor is in the path of this incident visible radiation, these emitted photons are absorbed and a ground-state electron residing in an s atomic orbital is promoted to a $3p$ "excited" atomic orbital and the transition for this is depicted as



This energy is absorbed and corresponds to

$$E_{3p} - E_{3s} = \Delta E = h\nu$$

This transition is also the most intense for Na among its other emission lines. In contrast to molecular spectra in solution, atomic spectra in the gas phase consist of very discrete and narrow wavelengths having very narrow bandwidths. For example, Zn has λ at 213.9 nm and a $\Delta\lambda = 0.002$ nm (104).

The AES and AAS determinative techniques can be furthered classified according to the excitation temperatures attained. The following is this classification for most optical, atomic spectral methods of elemental analysis:

Atomization	Atomization temp. (°C)	Basis	Acronym
Flame	1,700–3,150	Absorption	AAS
		Emission	AES
Electrothermal	1,200–3,000	Absorption	GFAA
Inductively coupled argon plasma	6,000–8,000	Emission	ICP–AES
Direct current argon plasma	6,000–10,000	Emission	DC P
Electric arc	4,000–5,000	Emission	Arc-source emission
Electric spark	40,000(?)	Emission	Spark-source emission

Of the six AES and AAS determinative instrumental techniques cited, the first three are most likely to be found in environmental testing labs and for this reason we will consider only these. Of the three, the two most important to TEQA are ICP–AES and GFAA. FI–AA is still important; however, its relatively high IDLs, particularly for the priority pollutant metals, serve to limit its usefulness. FI–AES remains the analytical determinative technique of choice for the quantification of alkali and alkaline-earth metal ions found in aqueous samples.

66. HOW DOES THE DESIGN OF AA, AE, AND AF INSTRUMENTS FUNDAMENTALLY DIFFER?

Figure 4.68 depicts the simplest instrument configurations for AE (top), for AA (middle), and for AF (bottom). Note how the positions of the flame source, optics, and detector differ among all three. The analyst should have this simple schematic in mind when approaching atomic spectroscopic instrumentation. With respect to the top schematic, instrumentation has advanced that have sought to maximize the emitted intensity of a particular wavelength while minimizing the background emission at that wavelength. For FI–AES or ICP–AES, it is the emitted line intensity of a particular wavelength against this background emitted intensity that forms the basis of TEQA. This includes the analysis of environmental samples for priority pollutant metal ions that follows the process of calibration, verification of the calibration [i.e., running sufficient ICVs (see Chapter 2)], interpolation of the calibration curve (quantitative analysis), and establishing the IDL for a given metal analyte. We proceed now to discuss ICP–AES.

67. WHAT IS ICP–AES?

This author has been searching for a good description of the ICP for this book! A recently written book, published by those affiliated with the Perkin-Elmer Corporation, at the time describes the ICP very well and we quote (105):

The ICP discharge used today for optical emission spectrometry is very much the same in appearance as the one described by Velmer Fassel in the early 1970's. Argon gas is directed through a torch consisting of three concentric tubes made of quartz or some other suitable material A copper coil, the load coil, surrounds the top end of the torch and is connected to a radio

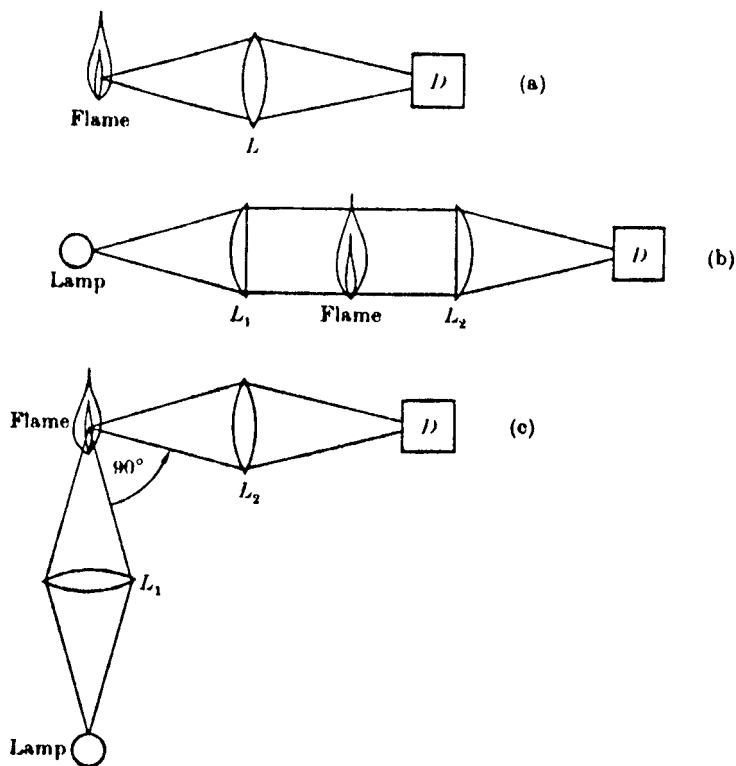


FIGURE 4.68 Schematics of the three simplest instrument configurations for AE, AA, and AF.

frequency (RF) generator When RF power, typically 700–1500 watts, is applied to the load coil, an alternating current moves back and forth within the coil, or oscillates, at a rate corresponding to the frequency of the generator. In most ICP instruments this frequency is either 27 or 40 MHz. This RF oscillation of the current in the load coil causes RF electric and magnetic fields to be set up in the area at the top of the torch. With argon gas being swirled through the torch, a spark is applied to the gas causing some electrons to be stripped from their argon atoms. These electrons are then caught up in the magnetic field and accelerated by them. Adding energy to the electrons by the use of a coil in this manner is known as inductive coupling. These high-energy electrons in turn collide with

other argon atoms, stripping off still more electrons. This collisional ionization of the argon continues in a chain reaction, breaking down the gas into a plasma consisting of argon atoms, electrons, and argon ions, forming what is known as an inductively coupled plasma discharge. The ICP discharge is then sustained within the torch and load coil as RF energy is continually transferred to it through the inductive coupling process. The ICP discharge appears as a very intense, brilliant white, teardrop-shaped discharge At the base, the discharge is toroidal, or “doughnut shaped” because the sample-carrying nebulizer flow literally punches a hole through the center of the discharge. The body of the “doughnut” is called the induction region because this is the region in which the inductive energy transfer from the load coil to the plasma takes place . . . the area from which most of the white light, the induction region, and into the center of the plasma gives the ICP many of its unique analytical capabilities.

Stanley Greenfield in England first published a report in 1964 on the use of an atmospheric pressure ICP for elemental analysis, whereas Velmer Fassel and colleagues made the earliest refinements including nebulization of the aqueous sample (106). The ICP–AES determinative method appeared in the early 1980s as EPA Method 200.7 for the determination of metals in drinking water. Figure 4.69 is a schematic from the original Fassel design and shows the three possible pathways up through the concentric cylindrical quartz “ICP torch.” An aqueous sample converted to an aerosol is taken up into the plasma via aerosol formation with Ar and any atomic emission due to the metals in the sample are observed atop the torch as shown. In an EPA contract lab that this author worked in during the 1980s, the ICP–AES instrument was operated continuously. A large tank containing liquified Ar serves well when continuous operation of the ICP torch is anticipated. In the 1990s, in academia, this author uses a compressed tank of Ar because of only intermittent use of the ICP–AES instrument. Figure 4.70 shows a simple schematic of a double-monochromator optical system that might be typical of mid-1980s technology for ICP–AES. The emitted radiation from the ICP is directed through a lens L and reflected off of a mirror M_1 . This reflected light enters slit S_1 and to mirror M_2 , which reflects the light to a diffracting grating, G, off of M_2 a second time. This reflected light exits through slit S_2 . A schematic for a typical ICP–AES is shown in Fig. 4.71.

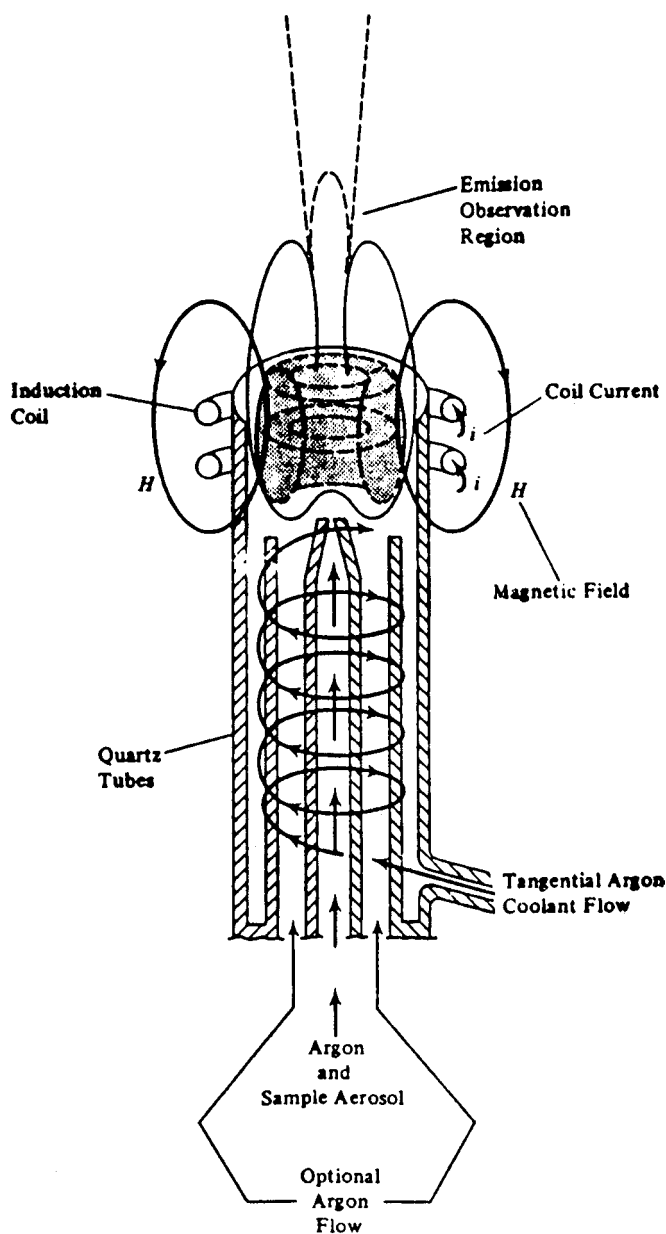
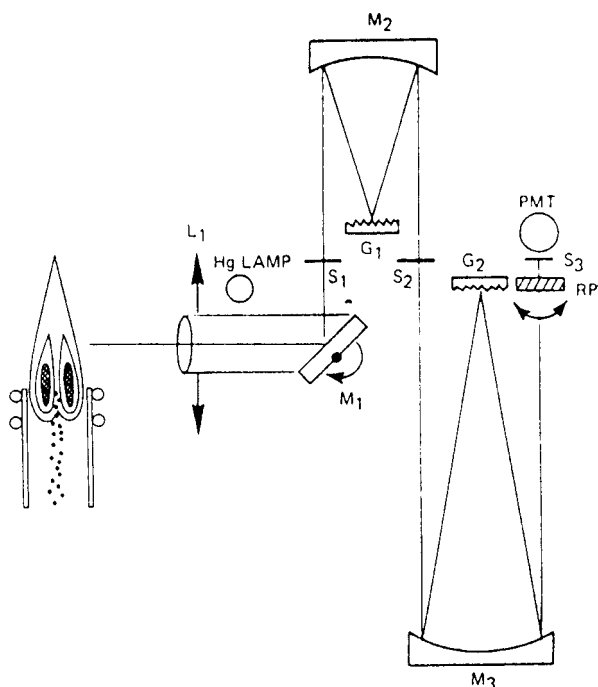


FIGURE 4.69 Schematic of the original Fassel design for ICP.



DOUBLE MONOCHROMATOR OPTICAL DIAGRAM

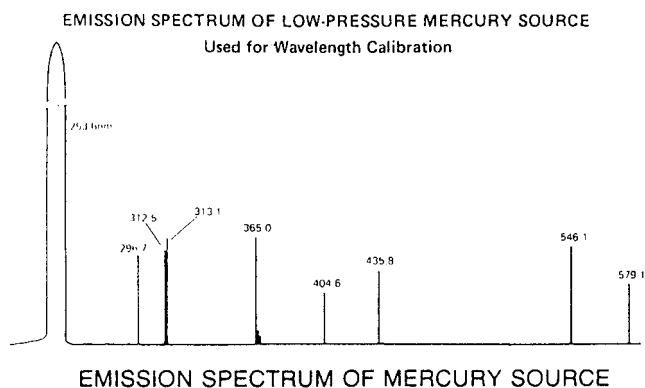
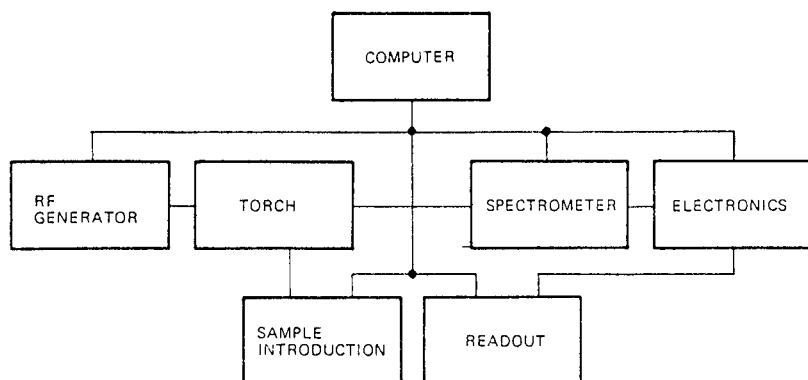
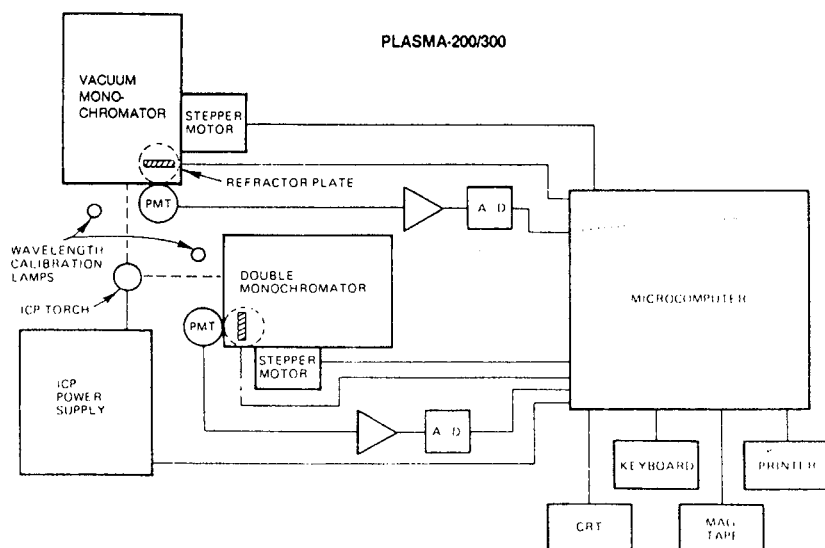


FIGURE 4.70 Schematic of a double monochromator for ICP-AES and emission spectrum of a mercury source.



BLOCK DIAGRAM OF BASIC INSTRUMENT DESIGN



BLOCK DIAGRAM OF PLASMA-200/300

FIGURE 4.71 Block diagrams of complete ICP–AES configurations.

68. HOW HAVE ICP–AES INSTRUMENTS EVOLVED?

Advances in design of ICP–AES instruments have led to systems that now incorporate an Echelle grating and a charged-couple detector as shown in Fig. 4.72 (110). A comparison of the major instrumental components for an ICP–AES for instruments manufactured in the 1980s versus those made in

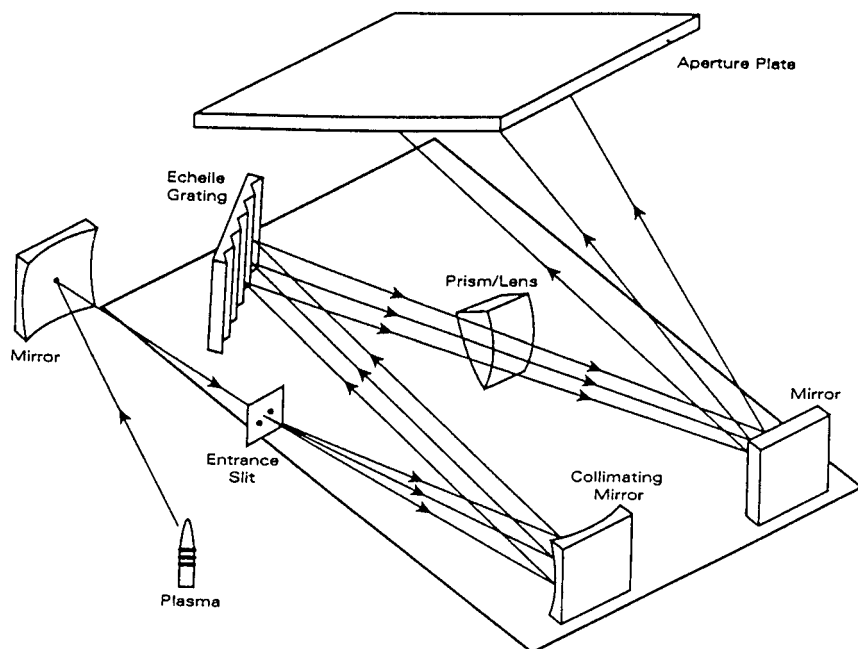


FIGURE 4.72 The Echelle grating used in contemporary ICP-AES instruments.

the 1990s, particularly during the latter part of the decade are shown in Table 4.20.

69. WHAT HAPPENS TO Ar AND TO A METAL ANALYTE IN THE PLASMA?

Electrons ionize the plasma gas according to



In addition, recombination can occur according to



with the formation of excited Ar atoms, symbolized by Ar^{*} , and a background emission at resonance lines of 104.8 and 106.7 nm respectively.

The plasma source serves two roles in ICP-AES:

TABLE 4.20 Comparison of ICP–AES Components Between Decades

Component	1980s	1990s
Sample introduction	Pneumatic	Pneumatic, frit, ultrasonic, direct insert, thermospray, electrospray
RF generator	Piezoelectric	Solid state
Torch	Greenfield or Fassel type	Modified Fassel type
Plasma view	Side-on (radial)	Side-on (radial), end-on (axial), dual
Monochromator	Czerney–Turner or Ebert	Echelle, dispersion of λ 's/prism, dispersion of diffraction orders
Detector	Photomultiplier	Solid state, charge transfer device
Signal processing	Analog, analog-to-digital, signal, then background	Analog-to-digital with storage of signal and background simultaneously

- To atomize the sample so as to free the metal analyte usually in their ground state
- To partially ionize the metal analyte with excitation of both atoms and ions

These processes are summarized as follows, where Ar^m represents a meta-stable atom (107):

Chief ionization processes	
Charge transfer	$\text{Ar}^+ + \text{M} \longrightarrow \text{M}^{+*} + \text{Ar}$
Electron impact ionization	$e^- (\text{fast}) + \text{M} \longrightarrow \text{M}^+ + 2e^- (\text{slow})$
Penning ionization	$\text{Ar}^m + \text{M} \longrightarrow \text{M}^{+*} + \text{Ar}$
Chief excitation processes	
Electron impact excitation	$e^- + \text{M} \longrightarrow \text{M}^* + e^-$
Ion–electron radiative recombination	$\text{M}^+ + e^- \longrightarrow \text{M}^* + h\nu$

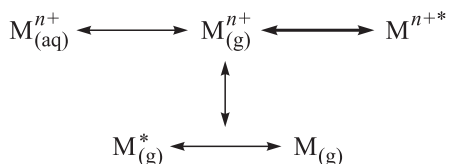
When the sum of the metal analyte’s ionization and excitation energies is below the ionization potential for Ar (i.e., ~16 eV), ionic lines become the most sensitive. The following table summarizes those elements that yield the most sensitive lines (107):

Elements whose ionic lines are most sensitive	Al, Ba, Be, Ca, Ce, Co, Cr, Fe, Hf, Hg, In, Ir, La, rare earths, Mg, Mo, Nb, Ni, Os, Pb, Sc, Sr, Ta, Th, Ti, U, V, W, Y, Zn, Zr
Elements whose atomic lines are most sensitive	Ag, As, Au, B, Bi, Ga, Ge, K, Li, Na, Rb, S, Sb, Se, Si
Elements whose atomic and ionic lines have similar sensitivities	Cu, Pd, Pt, Rh, Ni

70. CAN THE INTENSITY OF AN ATOMIC EMISSION LINE BE PREDICTED?

Yes, but we need to discuss relationships previously derived from statistical mechanics that relate to the population of atoms and ions among the various quantized electronic energy levels. Unless a solid sample is being directly introduced into the plasma by one of the direct insertion techniques, a metal ion dissolved in water, $\text{M}_{(\text{aq})}^{n+}$ is most likely to be found. This hydrated metal ion becomes “dehydrated” in the plasma, atomized, and then undergoes excitation and/or ionization at the extremely high tempera-

ture of the plasma. The use of double arrows below depicts the various equilibria occurring in the plasma:



The high temperature achieved in the ICP favors formation of analyte ions over formation of analyte atoms (90%; conversion to ions) as indicated by the thicker arrow.

The ratio of number of metal atoms that have their outermost electrons in the excited quantized energy level (N_{excited}) versus the number of atoms whose outermost electrons are in the ground state (N_{ground}) quantized energy levels and how this ratio is influenced by the temperature in the plasma are governed by the Boltzmann distribution. This well-known equation from statistical mechanics is

$$\frac{N_{\text{excited}}}{N_{\text{ground}}} = \left(\frac{g_{\text{excited}}}{g_{\text{ground}}} \right) e^{-\Delta E/kT}$$

where g_{excited} is the degeneracy of the excited state and g_{ground} is the degeneracy of the ground state. Degeneracy is the number of states available at each quantized energy level and $g = 2J + 1$, where J is the total angular momentum quantum number. ΔE is the energy-level spacing, k is Boltzmann's constant, (1.38×10^{-23} J/K or in terms of wavenumber, $k = 0.695 \text{ cm}^{-1}$) and T is the absolute temperature in degrees Kelvin. The total population among all quantized atomic energy levels is given by

$$N = n_0 + n_1 + n_2 + \cdots + n_i + \cdots$$

The total number of metal analyte atoms can be considered as being distributed among or partitioned into the various quantized energy states according to a partition function denoted by Z and can be written as

$$Z = g_0 + g_1 e^{-E_1/kT} + g_2 e^{-E_2/kT} + \cdots + g_i e^{-E_i/kT} + \cdots$$

The fraction of M atoms whose electrons are in the j th excited state becomes

$$\frac{n_j}{N} = \left(\frac{g_j}{Z} \right) e^{-E_j/kT}$$

The extent that M atoms are ionized in the plasma can be predicted from applying the Saha equation and after manipulation of the basic relationship, it be written as follows (108):

$$\frac{N_{ij}N_e}{N_{aj}} = \left(\frac{(2\pi m_e kT)^{2/3} 2Z_{ij}}{h^3 Z_{aj}} \right) e^{-E_j/kT}$$

These parameters are defined as follows:

N_{ij}	Number of ionic species j	E_j	Ionization potential of atomic species j
N_{aj}	Number of atomic species j	Z_{aj}	Partition function of atomic species j
N_e	Number of free electrons	Z_{ij}	Partition function of ionic species j
M_e	Mass of electron	Z_{aj}	Partition function of atomic species j
h	Planck's constant $h = 6.626 \times 10^{-34}$ J $s = 0.3336$ cm ¹ s	T	Ionization temperature
k	Boltzmann's constant		

Consider the transition between a ground-state atom whose potential energy is E_0 and an excited state j whose potential energy is E_j . The intensity of an emitted wavelength in the plasma depends on the following factors (109):

- The difference $E_j - E_0 = h\nu = \Delta E$, the number of metal atoms in the excited state, n_j
- The number of possible transitions between E_j and E_0 per unit time
- The Einstein transition probability A_j such that the emitted intensity I is proportional to the product of ΔE and A_j such that

$$I \propto \Delta E A_j$$

Upon solving the Boltzmann distribution for the number of atoms in the excited state, N_{aj} , and substituting for N_{aj} leads to the outcome of this section and answers the question posed:

$$I = \Phi \left(\frac{hcg_j A_j N}{4\pi\lambda Z} \right) e^{-\Delta E/kT}$$

where Φ is a coefficient to account for emission being isotropic over a solid angle of 4π steradians. For the AE intensity to be directly proportional to the number of ground-state atoms, N , as shown above, this requires that all other terms in the above equation remain constant. Therefore,

$$I \propto \text{conc}$$

and this relationship makes it possible to conduct TEQA!

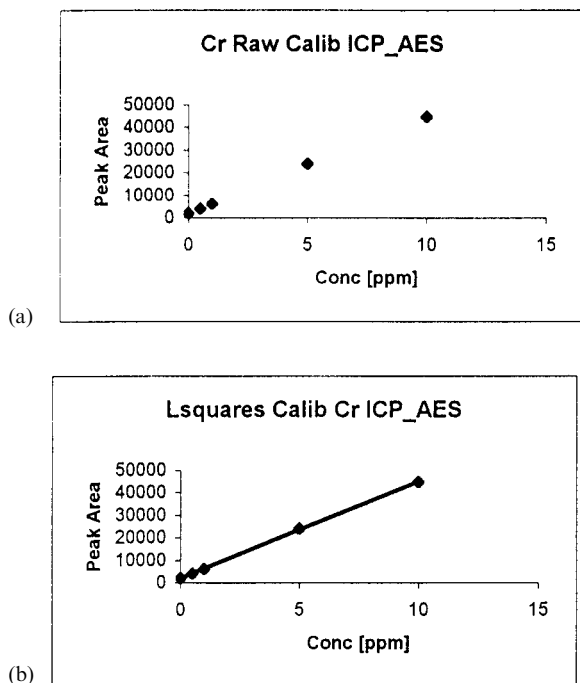
We assume for purposes of TEQA that a certified reference standard such as for Cr when aspirated into the plasma exhibits nearly identical parameters as that for Cr in the environmental sample. This requires good stability of the source characteristics (i.e., forward power and gas flow rates) (109). A calibration plot for Cr is shown in Fig. 4.73 and this plot was obtained using a Plasma 2000[®] ICP spectrometer (Perkin-Elmer) from the author's laboratory. The upper plot shows the raw calibration data using the common 267-nm line for Cr and the lower plot show how good these data points fit to a least squares regression line using the trendline in EXCEL[®]. The results shown below this plot were obtained from entering the calibration data into a computer program written by the author. IDLs were determined using principles discussed in Chapter 2.

71. WHY DO THE AE LINES BROADEN?

Two factors contribute to AE band broadening. The first is due to the motion of M atoms in the plasma, a so-called Doppler effect and the second is broadening due to collisions. Doppler broadening, symbolized by $\Delta\lambda_D$, depends on the wavelength chosen, the "kinetic temperature" and the molecular weight of the metal of interest and can be predicted as follows (109):

$$\Delta\lambda_D = 7.16 \times 10^{-7} \lambda \sqrt{\frac{T_{\min}}{M}}$$

Values for $\Delta\lambda_D$ are low for heavy elements (e.g. Au(II) at 200.08 nm has a value of 0.8 pm, whereas $\Delta\lambda_D$ values are high for light elements such as Be(II) at 313.11 nm that has a value of 5.9 pm. Collisional broadening results due to collisions among analyte ions, atoms, and neutral Ar atoms and has also been called pressure broadening. Doppler broadening is dominant near the center of the band, whereas collision broadening dominates near the tails. AE linewidths dictate what resolution is needed to resolve one AE emission line from another. For each transition metal, there is a plethora of lines to consider.



Slope of Least Squares Regression = 4289 counts /ppm Cr

y-intercept of Least Squares Regression = 2075 counts

correlation coefficient = 0.9998

standard deviation in the y-intercept = 193

y(critical) = 2965 counts

x(critical) = 0.21 ppm Cr direct aspiration of aqueous solution

x(detection) = 0.41 ppm Cr direct aspiration of aqueous solution

x(limit of quantitation) = 0.45 ppm Cr direct aspiration of aqueous solution

FIGURE 4.73 Calibration plots for the determination of chromium by ICP–AES showing (a) raw data and (b) a least squares regression fit to the raw data and statistical summary using LSQUARES.

We will not consider discussing the principles of ICP–MS here since not all environmental testing labs have such expensive instrumentation at present. You will most likely see atomic absorption spectrophotometers! In labs that have older ICP–AES instruments, EPA contract lab requirements are that the elements As, Se, Tl, and Pb be determined by graphite furnace atomic absorption and the remaining priority pollutant metals be determined by ICP–AES.

72. WHAT IS ATOMIC ABSORPTION?

It is the phenomenon by which resonance atomic emission lines are absorbed by atoms in the vapor phase; the phenomenon adheres to Beer's law and, hence, forms the basis for TEQA involving environmental samples that contain heavy metal residues. Kirchoff in the mid-1800s is credited with conceiving and performing the classical experiment that first observed atomic absorption. A simple schematic of the experiment is shown in Fig. 4.74. The yellow light emitted when a NaCl-coated nichrome wire is thrust into the flame was directed to a screen with a small hole. Observations of the brightness of the yellow light, reflected off of the solid back screen that disappears when elemental sodium was heated in a watch glass above a second burner, must have surprised Kirchoff! The resonance emission of the characteristic 589-nm yellow light was completely absorbed by the Na vapor, explaining the absence of light at the “detector” and was dubbed “atomic absorption.” Contemporary sources from resonance atomic emission lines of many elements emit in the colorless UV region of the electromagnetic spectrum. The photomultiplier tube (PMT)

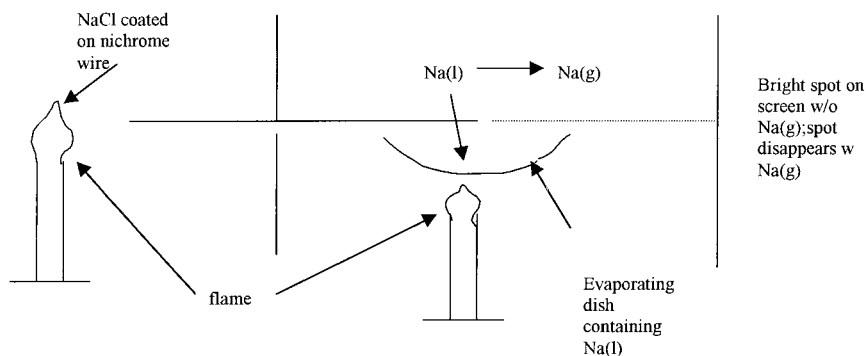


FIGURE 4.74 Schematic of the classic experimental configuration, first devised to observe the atomic absorption phenomenon.

can sense this “colorless” UV radiation enabling atomic absorption to “cast a wide net” to include most metallic and most metalloids like As and Se in the Periodic Table!

Atomic absorption as an analytical technique remained dormant until Walsh in Australia and Alkemade and Milatz in The Netherlands conceived the notion and published their findings in the same year, 1955 (110)! Varian Associates licensed the technology from Walsh and, to this day, Varian manufactures its AA instruments in Australia! The original experiment of Kirchoff is repeated every time an analyst performs a quantitative analysis of specific metal using an AA spectrophotometer! Significant modifications to the classical experiment have been made:

- The source of the resonance line has been replaced by hollow-cathode lamps (HCLs) or in some cases, electrodeless discharge lamps (EDLs)
- The second burner has been replaced by a concentric flow nebulizer with an acetylene–air or an acetylene–nitrous oxide burner
- The wavelength for the resonance line from the source is isolated from extraneous wavelengths due to interferences by a monochromator
- The back screen and eyeball replaced by photomultiplier tube and amplifying electronics!

This author has used AA spectrophotometers made by either Perkin-Elmer or Varian over the years. The advances in ICP–AES during the late 1970s and 1980s relegated FIAA to the “back burner” (no pun intended). The low IDLs enjoyed by contemporary GFAs have kept this technology relevant to the goals of TEQA. Before we focus our discussion on how GFAA is used to perform TEQA, let us compare AA instruments in general.

73. WHAT ELEMENTS OF THE AA SPECTROPHOTOMETER DO I NEED TO KNOW MORE ABOUT?

Single- versus double-beam design and the necessity for a HCL is the answer. Figure 4.75 is a schematic of a single-beam AA configuration showing the single beam of resonance line radiation emanating from a HCL, passing through a flame atomizer and into the entrance slit of a monochromator. Figure 4.76 shows how this basic configuration is modified to accommodate a double-beam configuration. A rotating chopper is a semitransparent device that directs a portion of the HCL resonance line radiation to become diverted, pass through the atomizer and on to the entrance slit to the monochromator, and comprise the sample beam while the transparent portion “bypasses” the atomizer directly to the monochro-

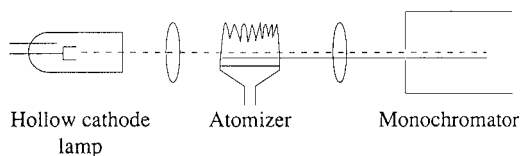


FIGURE 4.75 Schematic of a single-beam AA instrument. The dashed line represents modulated signal from the radiation source and the solid line represents direct current emission from the atomizer.

mator. The PMT in the double case alternatively “sees” sample intensity/reference intensity. This comparison yields a very stable noise level over time. When an aqueous solution is aspirated into the flame or when a microdrop is placed in the furnace, the transmitted power decreases in comparison to the reference power and a good signal-to-noise ratio can be realized. For this reason, double-beam AA instruments are preferred but are also more expensive. Figure 4.77 compares the major components of both a FIAA and a GFAA.

It would seem that an ordinary deuterium lamp that emits a continuum spectrum would make a low-cost radiation source for AA provided the source is passed through a monochromator whereby a narrow bandpass of wavelengths can be selected. However, in order for Beer’s law to be obeyed, the bandwidth at half height, $\Delta\lambda_{1/2}$ for the monochromator should be less than $\Delta\lambda_{1/2}$ for the analyte of interest—in this case, the atoms in the vapor phase of metallic elements. Robinson has articulated the problem this way (104):

With the use of slits and a good monochromator, the band falling on the detector can be reduced to about 0.2 nm. If a band 0.002 nm wide were absorbed from this, the signal would be reduced 1%. Since this is about the absorption linewidth of atoms, even with

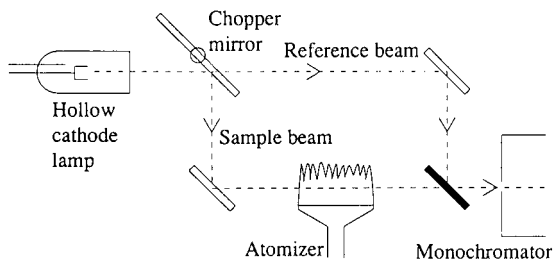
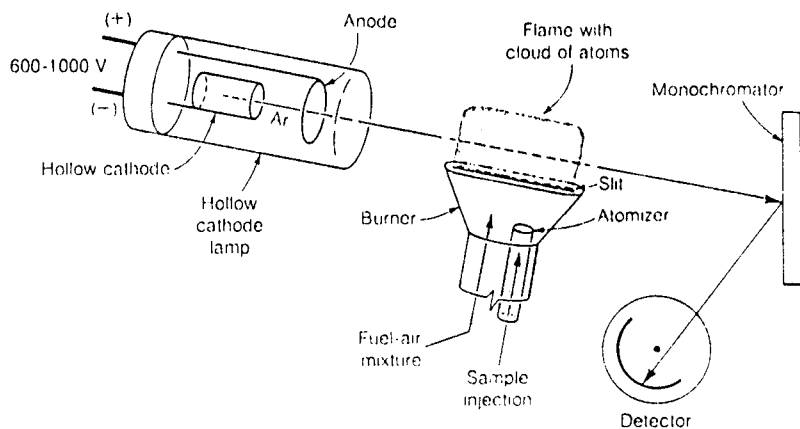
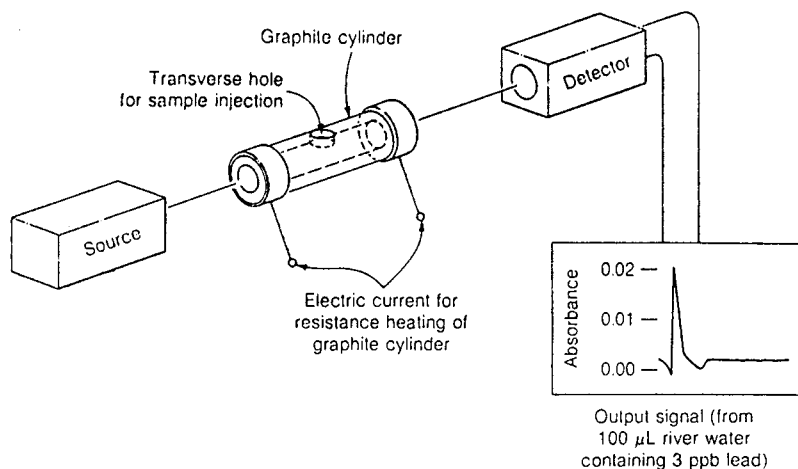


FIGURE 4.76 Light path in a double-beam AA design.



The major components of an atomic absorption spectrophotometer



Graphite furnace for atomic absorption analysis and typical output

FIGURE 4.77 Major components of FIAA and GFAA.

complete absorption of radiation by atoms, the total signal would change by only 1%. This would result in an insensitive analytical procedure of little practical use. The problem of using such narrow absorption lines was solved by adopting a *hollow cathode* as the radiation source.

74. WHY IS THE GFAA A SUITABLE TRACE DETERMINATIVE TECHNIQUE?

The relatively low IDLs listed in Table 4.19 suggest that GFAA is an ideal determinative technique for TEQA involving most metals. Contemporary GFAA instrumentation incorporates an autosampler that delivers a micro-drop of sample directly into the center of a cylindrically shaped graphite tube via a predrilled hole midway up the length of the tube. Because the total amount of sample is available and when combined with the ability of the instrument to emit the resonance line associated with the HCL or EDL and then absorb this narrow band of UV radiation across a path length of a few centimeters, an appreciable absorbance is measured from a very low analyte concentration. This is in contrast to the concentric flow nebulizer used in FIAA whereby most of the sample volume (~90%) is drawn to waste! For the highest-quality analytical determination, it is suggested that pyrolytically coated graphite tubes be used. Prior to use, a L'vov (after the Russian scientist) platform is inserted into the graphite tube in a manner shown in Fig. 4.78. The purpose of using a L'vov platform along with the additional expense is to isolate the sample from the tube walls to allow more reproducible atomization of the sample through indirect heating. The platform enables a higher atomization temperature to be reached as is shown in Fig. 4.79 and this produces more free atoms, which reduces interferences. Pyro-coated tubes can also be purchased with the platform already built in.

75. WHAT DETERMINES THE STRENGTH OF ABSORPTION FOR A GIVEN METAL ANALYTE?

In a manner similar to our discussion of atomic emission, the Boltzmann distribution again needs to be considered and the ratio of the number of

Atomic Absorption Spectroscopy

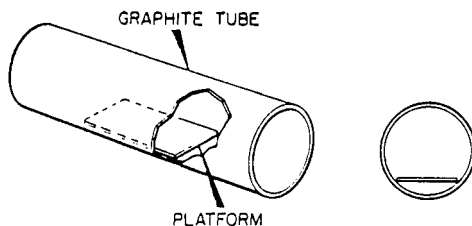


FIGURE 4.78 The L'vov Platform used in GFAA.

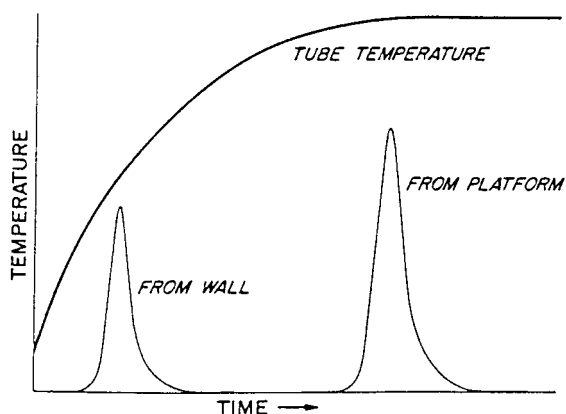


FIGURE 4.79 Effect of tube temperature with time on the atomization signal when sampling from the wall and L'vov platform.

atoms in an excited state to the number of atoms in the ground state is reiterated as follows:

$$\frac{N_{aj}}{N_0} = \frac{g_j}{g_0} e^{-E_{aj}/kT}$$

The maximum sensitivity obtained when 100% of all atoms are in their ground state (i.e., $N_{aj}/N_0 \sim 0$) and this ratio is low when the temperature is low and E_{aj} is low. Examples are as follows:

Element	λ (nm)	N_{aj}/N_0 at 3000 K
Cs	852.1	0.007
Zn	213.8	1×10^{-10}
Na	590	4×10^{-4}

Because most atoms are in their ground state, the atomic absorption signal depends on the number of ground state atoms, N_0 , and T has minimal influence unlike AES where changes in T cause significant changes in N_{aj}/N_0 as discussed earlier. Temperature-dependent chemical reactions may occur that influence the number of gaseous atoms formed and this leads to chemical interferences.

We proceed now to take the notion of atomic absorption for GFAA a bit further. (Benchmark papers are provided in Ref. 111.) A consideration of Einstein probability coefficients for the simple concept of a transition from

the ground electronic state to the first excited state results in a relationship between the absorbance A and more fundamental parameters assuming that $\Delta\lambda_{1/2}$ for the monochromator is less than $\Delta\lambda_{1/2}$ for the analyte of interest:

$$A = \frac{3.83 \times 10^{-13} f_{01} b N_0(t)}{\Delta\lambda_{\text{eff}}}$$

where f_{01} is the oscillator strength, b is the path length, $N_0(t)$ is the number of ground-state atoms in the atomic vapor at time t , and $\Delta\lambda_{\text{eff}}$ is the width of a rectangular absorption profile that has the same k area and peak value as the light sources. Oscillator strengths for selected metals are as follows:

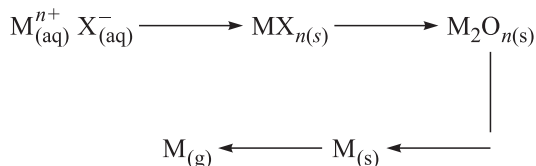
Element	λ (nm)	f
Ca	422.7	1.75
Cr	357.9	0.3
Cu	324.7	0.32
Na	589	0.57
	589.6	0.655
Tl	276.8	0.3
Zn	213.9	1.5

76. WHY IS IT IMPORTANT TO CORRECT FOR BACKGROUND ABSORPTION?

To achieve good precision and accuracy (see Chapter 2) in quantitating trace concentration levels of various metals of interest. Robinson has suggested that unknown samples, whose residual metal content is of interest to TEQA, the calibration standards, and the initial calibration verifications (ICVs) should be as “similar as possible” (113). To achieve this similarity the following considerations to both FIAA and GFAA become important:

- Use identical sample matrices for samples and standards
- Introduce a predominant anion such as sulfate or chloride at the same concentration in the matrix
- For FIAA, use the same fuel–oxidant mixture, sample pressure, and same flame height
- Use the same type of background correction technique

The metallic analyte is converted to atomic vapor in four steps:



Butcher and Sneddon describe the conditions for GFAA in a most interesting manner (112):

A graphite tube is typically 20 to 30 mm in length and 3 to 6 mm in diameter. The tube is surrounded by argon to prevent combustion in air at elevated temperatures. The sample is introduced into the furnace through a dosing hole (1–3 mm in diameter). In many cases, chemical compounds called chemical modifiers are also added to improve the sensitivity or accuracy for a given analyte. The temperature of the tube can be controlled from ambient up to approximately 2700°C, with heating rates up to 1500°C/s. It has been shown to be beneficial for many elements to insert a platform into the tube onto which the sample is placed . . . some metals may vaporize as a compound. The first step involves the relatively straightforward removal of solvent (usually water) from the sample. The remaining steps include chemical/physical surface processes, such as homogenous or heterogeneous solid–solid interactions, solid-phase nucleation and diffusion into graphite; heterogeneous gas–solid interactions, that is, adsorption/desorption and reaction of molecules with the wall to form atoms; homogeneous gas-phase reactions; and processes by which analyte leaves the furnace.

The purpose of background correction in AAS is to accurately measure the background and subtract this absorbance value from the uncorrected signal (signal + background) to give a “background-corrected signal.” This is accomplished in AAS instrumentation in one of three major ways:

- By continuum source background correction pioneered in 1965 by Koirtzmann and Pickett (see benchmark papers listed in Ref. 114)
- By self-reversal developed by Smith and Hieftje (114)
- Exploiting the discovery by Zeeman that an intense magnetic field causes atomic energy levels to split and this causes atomic lines to split into two or more components (114)

Several monographs give more comprehensive discussions of these three distinct approaches to background correction and are listed in Ref. 115.

To conclude our discussion on GFAA, as we did for ICP-AES, a calibration plot for the element Cr (as total chromium) is presented in Fig. 4.80 and is taken from the author's laboratory using the Model 310[®] (Perkin-Elmer) GFAA spectrophotometer. We also close our atomic spectroscopy discussion by summarizing in Table 4.21 the three major techniques to measure trace levels of metals from environmental samples and how to handle interferences. We now turn to two remaining determinative techniques of relevance to TEQA, infrared absorption spectroscopy, and capillary electrophoresis.

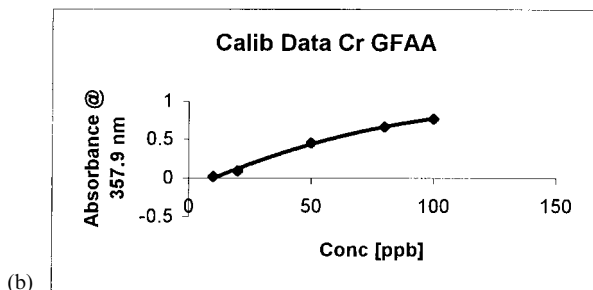
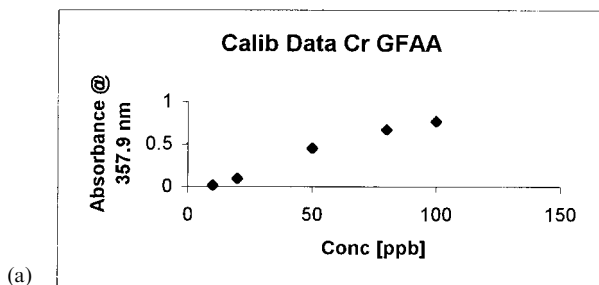
77. IN WHAT WAYS DOES IR ABSORPTION CONTRIBUTE TO TEQA?

Two principal applications of infrared (IR) absorption spectroscopy are useful to achieve the goals of TEQA and these include:

- The quantitative determination of the extent to which soil, groundwater, surface water, and wastewater are contaminated with oil and grease
- The quantitative determination of the extent to which an aqueous sample or aqueous soil leachate contains dissolved organic and inorganic forms of the element carbon

The hyphenated instrumental technique in which a GC was interfaced to a Fourier-transform infrared spectrometer (FTIR), via a gold-lined "light pipe" and given the acronym GC-FTIR gained widespread acceptance in analytical chemistry during the 1980s. IDLs for the GC-FTIR determinative technique are just not low enough to be of much use to TEQA. We will only discuss the principles of IR absorption spectroscopy insofar as this instrumental technique pertains to the two applications cited above. We will also not address the use of IR absorption spectroscopy in air analysis. A stand-alone IR spectrometer using a conventional dispersive monochromator or an interferometer will be discussed in light of these important determinative techniques. Nondispersive IR instruments for both oil and grease and for total organic carbon are quite common and will be briefly introduced.

Both determinative techniques cited above absorb radiation in the mid-IR region of the electromagnetic spectrum. The electromagnetic spectrum shown in Fig. 4.64 places IR absorption to the longer wavelength, lower frequency, and lower wave number in comparison to the visible region. IR radiation spans from just beyond the visible, known as the near-IR, beginning at $\lambda = 0.78 \mu\text{m}$ ($\bar{\nu} = 13,000 \text{ cm}^{-1}$), though the mid-IR,



Slope of Least Squares Regression = 0.0087 counts /ppb Cr

y-intercept of Least Squares Regression = 0 counts

correlation coefficient = 0.9905

standard deviation in the y-intercept = 0.0436

y(critical) = 0.1086 counts

x(critical) = 18.7 ppb Cr direct injection of aqueous solution

x(detection) = 35.8 ppb Cr direct injection of aqueous solution

x(limit of quantitation) = 69.3 ppb Cr direct aspiration of aqueous solution

FIGURE 4.80 Calibration plots for the determination of chromium by GFAA showing (a) raw data and (b) a least squares regression fit to the raw data and statistical summary using LSQUARES.

2.5–50 μm ($\bar{\nu} = 4000\text{--}200\text{ cm}^{-1}$), and, finally, to the far-IR beginning at $\lambda = 40\text{ }\mu\text{m}$ ($\bar{\nu} = 250\text{ cm}^{-1}$) and ending at $\lambda = 40\text{ }\mu\text{m}$ ($\bar{\nu} = 10\text{ cm}^{-1}$).

Historically, mid-range IR absorption spectroscopy has been a valuable qualitative instrumental technique and has served the science of organic chemistry very well. The mid-IR spectrum is a qualitative property of an

TABLE 4.21 Summary of Physical and Chemical Interferences in Atomic Absorption and Emission Spectroscopy

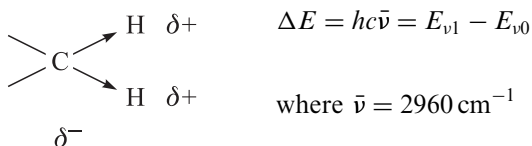
Atomic spectral technique	Type of Interference	Remedy
Flame AA	Ionization	Add a buffer to suppress
	Chemical	Add a releasing agent
Graphite furnace AA	Physical	Use a $C_2H_2-N_2O$ flame
	Physical	Dilute the sample, match the matrix, and use the standard addition mode of instrument calibration
	Physical	Adjust furnace temperature conditions
ICP-AES	Molecular absorption	Zeeman or D_2 background correction
	Spectral	Zeeman background correction
	Spectral	Use alternative AE line or subtract out background
	Matrix	Use the internal standard mode of instrumental calibration

organic compound and provides important structural information. For example, if you want to distinguish between an aliphatic and an aromatic hydrocarbon, a mere record of their respective IR spectra, particularly around 3000 cm^{-1} , reveals the difference! Those interested in a more in-depth introduction to IR spectrometry from the organic chemist's viewpoint should consult Shriner et al. (116).

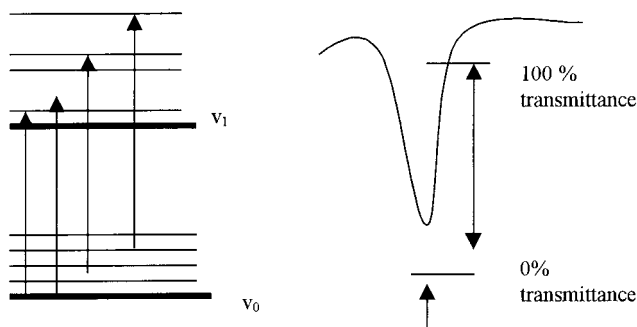
Photons whose λ 's fall to within the mid-IR region of the electromagnetic spectrum are absorbed by organic molecules in both the solid and liquid phases and yield a characteristic absorption spectrum. If the photons of emitted IR radiation match the quantized energy level spacing between the ground-state vibrational level denoted by v_0 , and the first excited vibrational level denoted by v_1 , a fundamental absorption is said to have occurred at that wavelength or wave number. IR absorption from the sun by glass windows in a closed automobile is a common example of how infrared radiation heats the atmosphere and this phenomenon has helped coined the term "greenhouse" effect. Herschel performed a similar experiment in 1800 when he directed sunlight through a glass prism to a blackened thermometer as a detector and thereby now is credited with the discovery of IR.

78. HOW DO I QUANTITATE OIL AND GREASE IN WASTEWATER USING IR ABSORPTION?

We already discussed the nature of sample preparation for this straightforward, yet elegant, quantitative determination in Chapter 3. Both aliphatic and aromatic hydrocarbons (HCs) containing many carbon to hydrogen covalent bonds are present in oil and grease. Suppose some motor oil is found dispersed into surface water due to some type of spill. Let us assume that a sample of this wastewater has undergone either a LLE or an SPE and the recovered HCs are now dissolved in a solvent that lacks the C—H bond such as 1,1,2-trichloro-trifluoroethane. (TCTFE). The C—H bond possesses a dipole moment due to the slight difference in electronegativity between the elements carbon and hydrogen that changes upon absorbing IR radiation between 3200 and 2750 cm^{-1} in the mid-IR region. One such mode of vibration that absorbs IR radiation in this region is known as symmetric stretching and is depicted as follows:



The energy level spacing, ΔE , between the ground-state vibrational level and first excited-state vibrational level is depicted as follows along with a typical IR absorption band for the symmetric stretch just discussed:



The fact that numerous quantized rotational levels exist within each vibrational level serves to help explain the broad-band nature of infrared spectra along with the fact that samples are in condensed phases.

In contrast to UV-vis stand-alone spectrophotometers, dispersive IR spectrometers are designed so that the sample is positioned immediately after the radiation source and just before the dispersive device. This is not the case for a FTIR spectrometer. To assist in distinguishing between dispersive and FTIR instruments, a schematic of the two is presented in Fig. 4.81. In a review article in 1968, Whetsel declared that “as far as instrumentation is concerned, I know of no major new developments on the horizon” (117). Then came the revolutionary development of FTIR spectroscopy based on the Michelson interferometer (he received the 1907 Nobel Prize for this invention). FTIR designs have all but displaced dispersive designs in infrared spectral technology today. However, the analyst is likely to find dispersive IR instruments that are dedicated to trace oil and grease determinations in environmental testing labs today. Quantitative IR analysis, as is true for UV-vis and atomic spectroscopy, is based on Beer’s law. The experiment introduced in Chapter 5 provides sufficient detail to quantitatively determine the concentration of oil and grease in an environmental sample. Several texts provide good introductory discussions on IR absorption spectroscopy including FTIR (118). This author has available to him a Model 1600 FTIR spectrometer (Perkin-Elmer) and this instrument utilizes software developed by Perkin-Elmer consistent with ASTM Method D 3921 and similar to EPA Method 431.2 (Oil and Grease, Total Recoverable) and Method 418.1 (Petroleum Hydrocarbons, Total Recoverable). The idea that a nonpolar extracting solvent that lacks the C—H stretch in the mid-IR

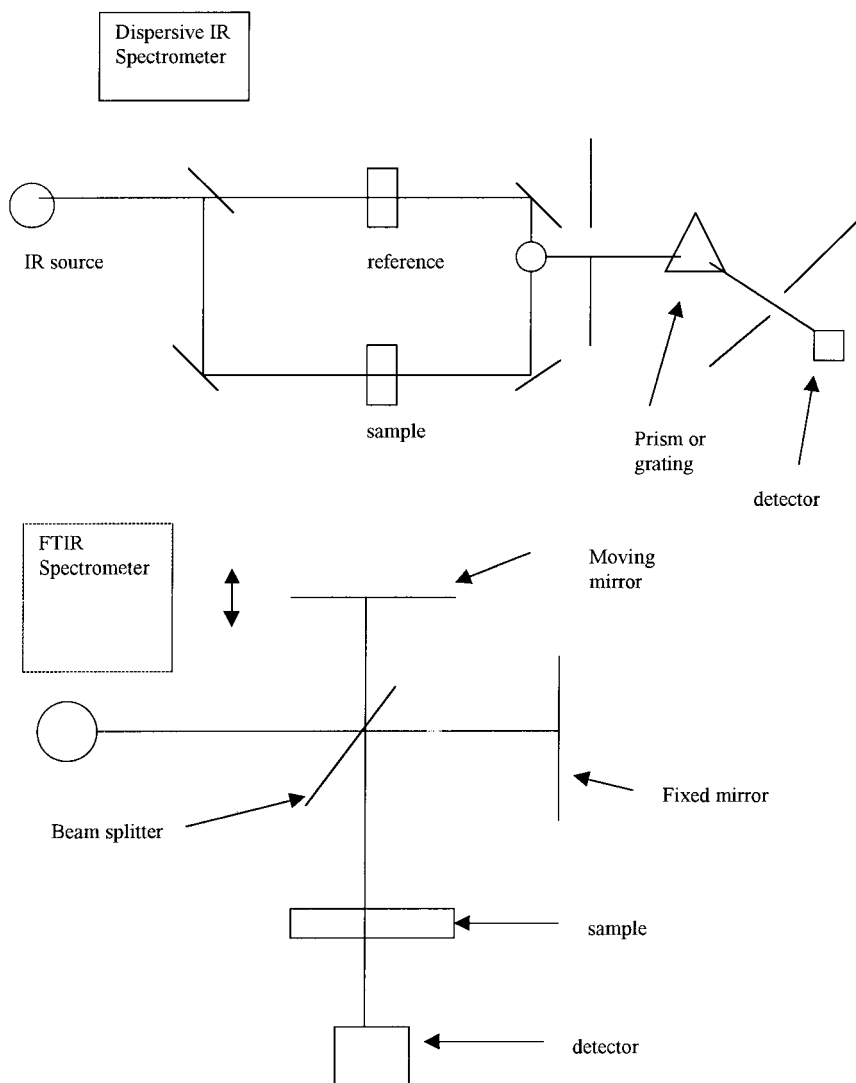


FIGURE 4.81 Comparison of schematics between a dispersive IR and FTIR spectrometer.

originated from early work published by Gruenfeld at EPA (119). That portion of the IR spectrum between 3200 and 2750 cm^{-1} of iso-octane dissolved in TCTFE is shown in Fig. 4.82. This portion is sufficiently well resolved that distinct C—H stretching absorption bands can be seen for this group frequency. Almost 95% of all absorption in the region between

3100 down to 2700 cm^{-1} is due to C—H stretching and includes the following:

- Asymmetrical stretching of a C—H bond in both a methyl and a methylene group. Both are present in iso-octane and centered at 2962 cm^{-1} .
- Symmetrical stretching of a C—H bond in a methyl group centered at 2872 cm^{-1} .
- Asymmetrical C—H stretching present in a methylene group at 2926 cm^{-1} .
- Symmetrical C—H stretching present in a methylene group at 2853 cm^{-1} .

A horizontal baseline is drawn as shown above to define a 100% T . The % T for an absorption as shown above is measured at the minimum of the absorption band. Beer's law is used in a manner similar to that for UV-vis spectroscopy and the measured % T is related to the absorbance according to

$$A = 2.0 - \log(\%T) = \epsilon bc$$

Quantitative IR therefore requires a cuvette of fixed path length b . It so happens that the standard rectangular quartz cuvette absorbs everywhere in the mid-IR except in the C—H stretching region! This author, using a Model 567 Grating IR spectrometer (Perkin-Elmer) and a 10-mm rectangular quartz cuvette developed the data and external calibration for petroleum hydrocarbons as shown in Fig. 4.83 using data shown in Table 4.22. We have used the same EXCEL programming to generate the raw data

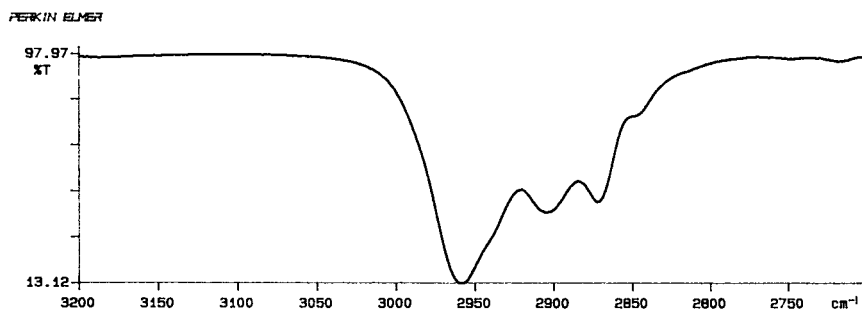


FIGURE 4.82 The IR spectrum over the wave-number range from 3200 to 2750 cm^{-1} for iso-octane dissolved in TCTFE from a Model 1600[®] (Perkin-Elmer) FTIR spectrometer.

calibration points in a manner similar to what we did for the GFAA and ICP–AES data discussed earlier. The analytical results for the unknown samples are real and were reported at the time. Note the influence of the spiked Soil A in comparison to the unspiked Soil A. Results for Soils B and C are given for comparison and these results serve to illustrate the utility of IR absorption spectroscopy toward achieving yet another goal of TEQA. One other determinative technique that utilizes IR absorption needs to be

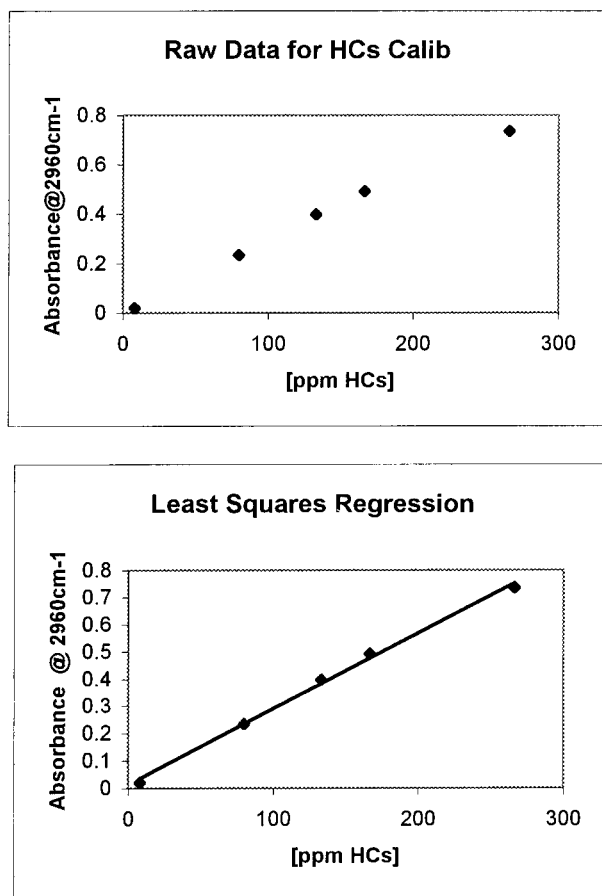


FIGURE 4.83 Calibration plots for the determination of petroleum hydrocarbons by FTIR showing raw data and a least squares regression fit to the raw data.

TABLE 4.22 Calibration Data and Quantitative Analysis for Unknown Samples for Trace Petroleum Hydrocarbons in Water

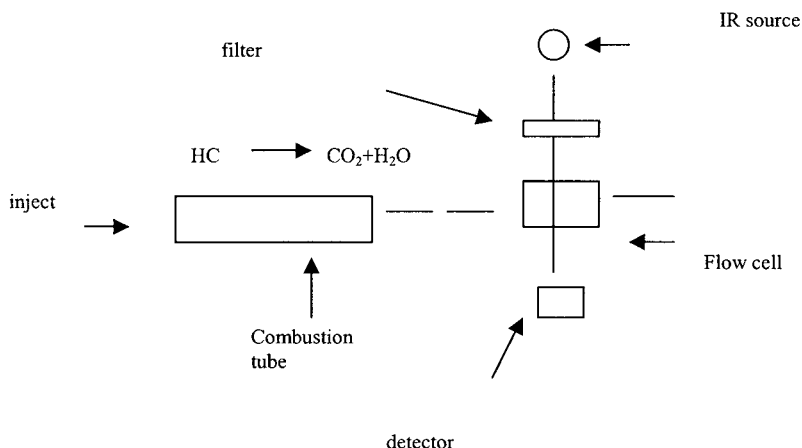
Standard No.	Concentration of HC (ppm)	Absorbance at 2960 cm ⁻¹
1	7.99	0.021
2	79.9	0.235
3	133.2	0.492
4	266.4	0.735
Unknown Sample	Found	Measured
EPA reference	199.3	0.596
Blank	0.8	0.014
Groundwater	28.1	0.09
Soil A	23.0	0.076
Soil A spiked with HCs	213.7	0.607
Soil B	30.6	0.097
Soil C	21.2	0.071

addressed and that is the determination of total organic carbon, whose acronym is TOC.

79. WHAT IS TOC AND HOW IS IR ABSORPTION USED?

The TOC content of a sample of water is an important and non-compound-specific parameter. It is measured by oxidizing the dissolved organic carbon (DOC) and quantitating the evolved carbon dioxide. One way to do this is to sweep the combustion products into a nondispersive IR photometer (NDIR) that has been tuned to measure CO₂. This is accomplished by measuring the absorbance of the characteristic C—O stretching vibration. Because instrumentation designed for this application responds to any and all sources of carbon in the sample, to measure TOC requires that the inorganic carbon content be removed from the sample. Some instruments measure the TIC (total inorganic carbon) content as well and arithmetically subtract this TIC value from a the total carbon (TC) measurement. Carbonates and bicarbonates are the principal constituents of the TIC content in an environmental sample. These anions can be converted to carbon dioxide by acidifying the aqueous sample, then purging the sample with inert gas. This leaves the sample with a level of dissolved organic carbon. In a manner that is similar to a gas chromatograph, the aqueous sample is injected into a carrier stream and swept directly into a high-temperature

combustion tube. A schematic of a TOC analyzer that incorporates a high-combustion quartz tube such as the Model 915B (Beckman Instruments) and satisfies Method 5310B (Standard Methods) is as follows:



A more recently manufactured instrument, available in the author's lab, the Model 1010 TOC Analyzer (O-I Analytical), utilizes Method 5310D (Standard Methods for the Examination of Water and Wastewater) in the instrument design to first remove inorganic carbon via acidifying with phosphoric acid followed by purging with inert nitrogen gas. An oxidizing reagent, potassium persulfate, is then added to the reaction vessel and the vessel is heated to 100°C to promote complete oxidation of organic carbon to CO₂. The CO₂ gas is removed from the reaction vessel with purge gas and swept to the NDIR.

80. WHAT IS CAPILLARY ELECTROPHORESIS AND WHAT ROLE DOES IT HAVE IN TEQA?

We next consider a determinative technique introduced during the late 1980s and commercialized during the 1990s to the arsenal of instrumental analysis relevant to TEQA—capillary electrophoresis (CE). Our discussion of CE forces us to return to the separation sciences. CE encompasses a number of distinct modes:

- Capillary zone electrophoresis (CZE)
- Capillary gel electrophoresis
- Micellar electrokinetic capillary chromatography (MEKC)

- Capillary electrochromatography (CEC)
- Capillary isoelectric focusing
- Capillary isotachopheresis

Li has provided a good definition of CE (120):

In electrophoresis, a mixture of different substances in solution is introduced, usually as a relatively narrow zone, into the separating system, and induced to move under the influence of an applied potential. Due to differences in the effective mobilities (and hence migration velocities) of different substances under the electric field, the mixture then separates into spatially discrete zones of individual substances after a certain time.

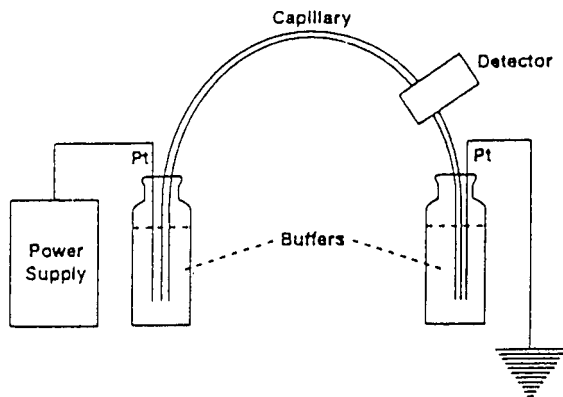
Our focus will be on CZE in this book although MEKC holds great potential for selective organic priority pollutant trace analysis. After we introduce the underlying principles of CZE, we will cite this author's efforts in developing a method to separate inorganic anions.

It is useful to compare CE with the two established instrumental separation techniques already discussed, namely HPLC and GC. The three schematics shown in Fig. 4.84 provide a means to compare and contrast this third contributor to separation science. Moving from left to right across all three schematics reveals the following:

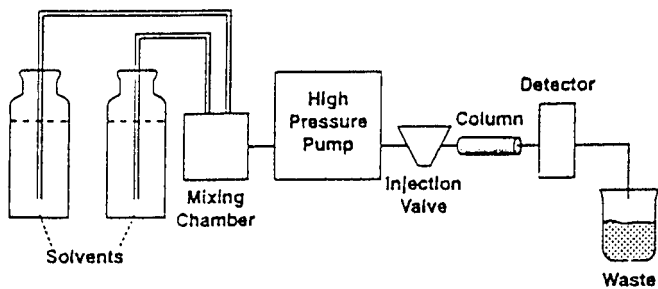
- All three techniques require a source of high potential energy. For CE, a high-voltage power supply is required; for HPLC, a fluid at high pressure is required; for GC, a compressed gas at high pressure is required.
- All three techniques require a column within which the separation of specific chemical compounds can be achieved. For CE, an uncoated narrow-bore cap column; for HPLC, a packed column; for GC, a coated-cap column or packed column.
- All three techniques require some means to detect and to quantitate the separated chemical compounds. For CE, an UV absorbance detector was first used; for HPLC, several detectors as already discussed; for GC, several detectors as already discussed.

Electrophoresis has been known since 1886 when Lodge observed the migration of protons, H^+ , in a tube of phenolphthalein dissolved in a gel; Hjerten in 1967 first used a high electric field in solution electrophoresis using a 3-mm-i.d. capillary. However, Jorgensen and Lucas are credited with writing the benchmark paper that demonstrated highly efficient separations using narrow-bore ($< 100\ \mu\text{m}$ i.d.) capillaries (121). Figure 4.85 vividly depicts, in a somewhat oversimplified manner, the basic components of a CE instru-

A



B



C

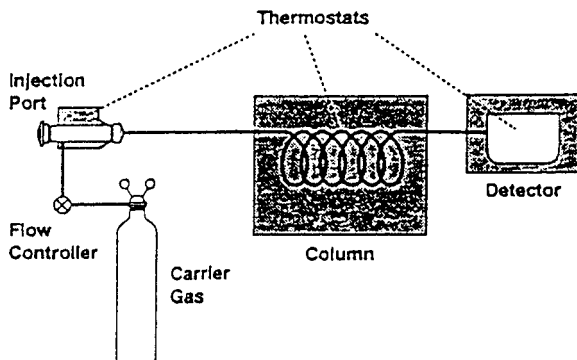


FIGURE 4.84 Comparison of CE (A) and HPLC (B) and GC (C).

ment. A CE instrument can be built from simple components as shown in the figure and consists of two beakers joined by a filled capillary column. A high-voltage power supply that is capable of providing 30,000 V is impressed onto Pt electrodes that are placed in each beaker. Due to this extremely high voltage requirement, an open configuration such as is shown in Fig. 4.85 is very dangerous! Safety considerations have led to the development of instruments that provide the necessary protection so that CE can be safely performed. As has been true in the historical development of GC and HPLC, the number of manufacturers of CE instruments rose and fell during the late 1980s and early 1990s and, today, only a few manufacturers remain. We digress briefly to introduce the basic theory underlying CE by asking the following question.

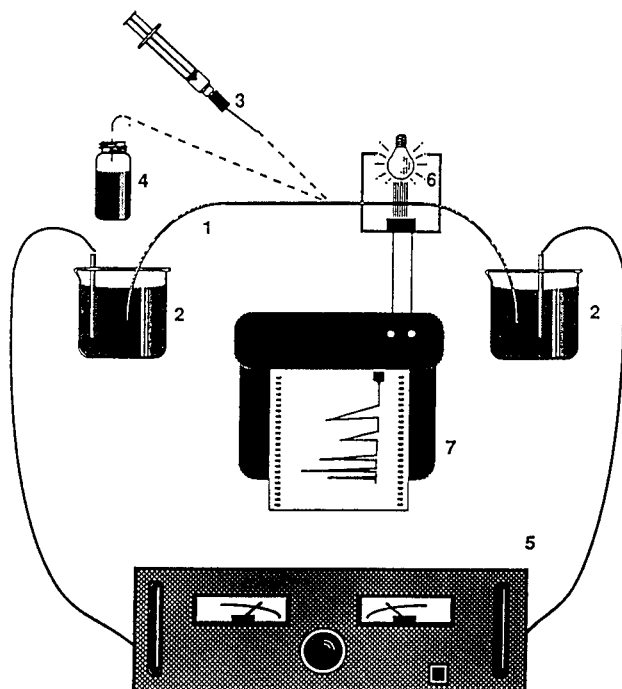


FIGURE 4.85 Basic components of a CE system: (1) fused-silica capillary; (2) electrolyte vessels with electrodes; (3) syringe-to-capillary adaptor (replaced in commercial instruments by pressure or vacuum-driven rinse); (4) sample vial raised to a level necessary for sample introduction by hydrostatic pressure; (5) regulated high-voltage power supply; (6) detector; (7) data acquisition device.

81. WHAT FACTORS INFLUENCE SEPARATIONS IN CE?

Applying such a large voltage across a capillary tube that is filled with electrolytes actually moves the fluid from anode to cathode and this phenomenon is termed electro-osmosis. CE opens up the possibility to quantitate the presence of analytes of interest to TEQA that are either cationic, anionic, or neutral. Cations migrate to the cathode, ahead or behind the electro-osmotic velocity, depending on the polarity of the electrodes. Anions migrate to the anode, ahead or behind, and neutrals migrate with the electro-osmotic force. Electro-osmotic flow originates from the negative charges on the inner wall of the capillary tube due to deprotonizing surface silanol groups in neutral or alkaline medium. Cations from the buffer move toward this negatively charged surface. It is the water dipoles that surround the cation that get “dragged” when the electric field is applied.

To better explain electro-osmosis from the perspective of the analyte, consider the following analogy. What happens to the velocity (i.e., from physics, speed *and* direction) of an individual when he/she “rides the horizontal escalator” like those found in airport terminals? The velocity of the escalator is analogous to what is called the electro-osmotic mobility, symbolized by μ_{EO} , and the velocity of the individual riding the escalator is analogous to the electrophoretic mobility, symbolized by μ_{EP} . When you walk in a direction that is in the same direction as that of the horizontal escalator, your speed relative to a stationary observer standing on the floor next to the moving escalator is greatly increased. If you walk in a direction 180° opposite the direction of the escalator, you will still be carried in the direction of the escalator with respect to the stationary observer. You will, however, arrive at the end of the ride at a much later time! The vector notation for the mobilities of cations and anions is as follows:

—————→ μ_{EO}

—————→ μ^+

←———— μ^-

The net effect to the detector (our stationary observer) is as follows:

+ —————→

0 —————→

- —————→

Ions originating from a buffer also have an electrophoretic mobility, symbolized by μ_{ion} . It should also be appreciated that the fluid flow profile through a tube is flat when the driving force is an electric field versus the parabolic profile for flow created by a pressure difference.

The derivation shown below is adapted from that introduced earlier (122). The electro-osmotic velocity, v_{EO} , is defined at the product of the electro-osmotic mobility, μ_{EO} , and the applied electric field, E . For an applied voltage V and for a length of capillary L , we can write

$$v_{\text{EO}} = \mu_{\text{EO}}E = \mu_{\text{EO}} \frac{V}{L}$$

Similarly, the electrophoretic velocity, v_{EP} , is related to the electrophoretic mobility, μ_{EP} , according to

$$v_{\text{EP}} = \mu_{\text{EP}} \frac{V}{L}$$

The magnitude of μ_{EP} is related to the charge-to-size ratio of the analyte ion. The time it takes for an analyte once introduced into the capillary inlet to the time detected is known as the solute migration time t . The solute migration time is unique to a given analyte, and much like the solute retention time, t_R , in GC and in HPLC, t is defined as follows and, in turn, can be written in terms of mobilities:

$$t = \frac{L}{v_{\text{EP}} + v_{\text{EO}}} = \frac{L^2}{(\mu_{\text{EP}} + \mu_{\text{EO}})V}$$

The above equation demonstrates that the CE solute migration time depends on the following:

- Solute charge to size ratio (i.e., μ_{EP})
- Applied voltage, V
- Capillary wall surface chemistry (i.e., μ_{EO})
- Buffer composition and concentration
- Length of column from inlet to detector, L

The only factor contributing to band broadening is, unlike HPLC, longitudinal diffusion. The spread in the the axial direction down the center of the capillary for ions and molecules introduced as a tight plug at the inlet to the column can be described by the Einstein equation:

$$\sigma^2 = 2Dt$$

where D is the molecular diffusion coefficient. The column efficiency, defined in terms of the number of theoretical plates, N , as we did in chromatography, is related to the capillary length, L , and the spread of molecules due to longitudinal diffusion of analyte through the capillary column, denoted by σ_L :

$$N = \frac{L^2}{\sigma_L^2} = \frac{(\mu_{EP} + \mu_{EO})V}{2D}$$

The above equation suggests that the efficiency in CZE is related to the applied voltage and not capillary column length. Maximize efficiency and a reduction in analysis times is obtained using high voltages and short columns. This can be accomplished only by efficient heat dissipation. Control of column temperature in CE becomes very important.

To answer the question posed, as we did for GC and HPLC, we must consider those factors that influence the electrophoretic resolution R_S . R_S can be related to the column efficiency N and the relative velocity difference between two zones, denoted by $(\Delta v/\bar{v})$ and this relationship is

$$R_S = \frac{1}{4}\sqrt{N}\left(\frac{\Delta v}{\bar{v}}\right)$$

This equation can be restated in terms of electrophoretic and electro-osmotic mobilities as follows:

$$R_S = \frac{1}{4}\sqrt{N}\left(\frac{\mu_{EP2} - \mu_{EP1}}{\bar{\mu}_{EP}}\right)$$

where a mean electrophoretic mobility between separated peaks 1 and 2 is given by a simple average, $(\bar{\mu}_{EP})$, over the mobility of each analyte according to

$$\bar{\mu}_{EP} = \frac{1}{2}(\mu_{EP1} + \mu_{EP2})$$

Eliminating N in the above equation in terms of electrophoretic ion mobilities yields

$$R_S = 0.18(\mu_{EP2} - \mu_{EP1})\left(\frac{V}{D(\bar{\mu}_{EP} + \mu_{EO})}\right)^{1/2}$$

This equation can be further simplified to yield:

$$R_S = \frac{1}{4} \left(\frac{V}{2D} \right)^{1/2} \frac{\Delta\mu_{EP}}{(\bar{\mu}_{EP} + \mu_{EO})^{1/2}}$$

Electrophoretic resolution, R_S , is found to depend on the following:

- $\Delta\mu_{EP}$, the difference in electrophoretic mobility between zones 1 and 2
- V , the applied voltage
- μ_{EO} , the electro-osmotic mobility
- $\bar{\mu}_{EP}$, the mean electrophoretic mobility of peaks 1 and 2

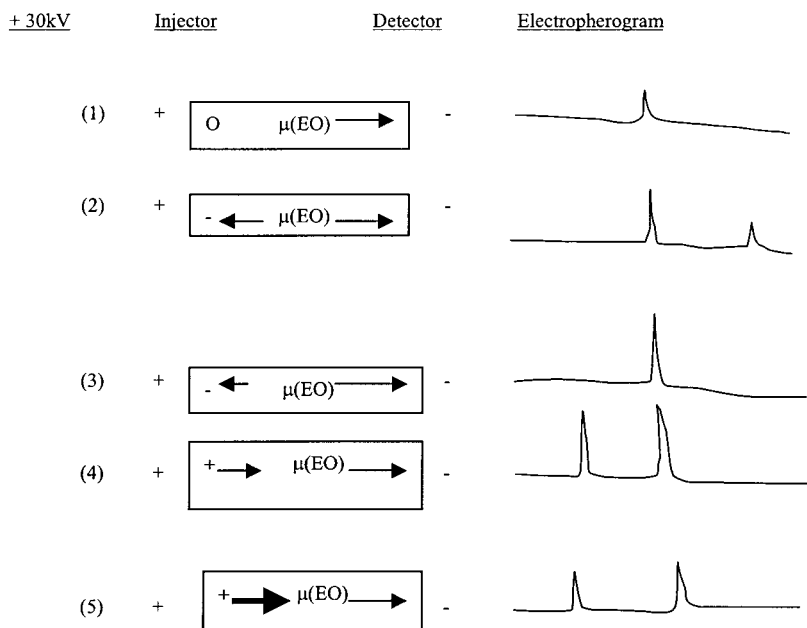
The practice of CZE is largely influenced by the following factors:

- The chemical nature of the buffer, the pH of the buffer, and the concentration of buffer
- Ionic strength
- Temperature, quite sensitive, need to keep constant
- Viscosity
- Dielectric constant
- Applied voltage
- Length of capillary between the inlet and detection
- Capillary diameter, significant factor with respect to Joule heating, column diameters $< 100 \mu\text{m}$ i.d. minimizes Joule heating
- Chemical nature of buffer additives such as the % organic modifier, buffer ions of different μ_{EO}
- Injection time
- Internal capillary-wall surface chemistry

82. HOW DOES ELECTRODE POLARITY INFLUENCE CE?

Commercially available CE instruments are capable of placing either a positive or negative charge on the Pt wire at the injector and at the same time placing a negative or positive charge on the Pt wire at the detector, respectively. A positive or negative potential can be programmed in via microprocessor. Depicted below is a schematic showing the various cation, anion, and neutral solutes introduced into a capillary along with the anticipated electropherograms to the right for impressing $+30 \text{ kV}$. In case 1, a neutral organic compound that absorbs UV radiation (this assumes that the CE instrument incorporates a UV absorption detector) is introduced into the injector end of a hypothetical column. This neutral “marker” will migrate with the same velocity as that of the

electro-osmotic force and arrive at the detector end with a migration time that corresponds to the electro-osmotic velocity. Any anions that are present reach the detector after the neutral marker, depending on the magnitude of their anion electrophoretic mobility as shown in cases 2 and 3. Cations that are present in the sample migrate ahead of the neutral marker, depending on their respective cation mobilities, as shown in cases 4 and 5. Cases 4 and 5 are illustrated in Fig. 4.86, in which three protonated peptides are separated. This electropherogram was obtained in the author's lab using a Model 270A-HT (Applied Biosystems, now called PE-Biosystems). These schematics are as follows:



Impressing -30 kV to the injector/detector Pt wires reverses the polarity and drastically alters the order of ion migration as shown in the second scenario depicted below. The detector is now anodic and the injector becomes cathodic. Case 1 shows the migration time for a neutral marker. Cases 2 and 3 depict two cations of different μ_{EP} 's. Cases 4 and 5 show the expected migration times for two anions with different values of μ_{EP} . To illustrate the use of a reversed polarity, consider the electropherogram, also obtained in the author's lab, shown in Fig. 4.87. A mixture of the sodium or

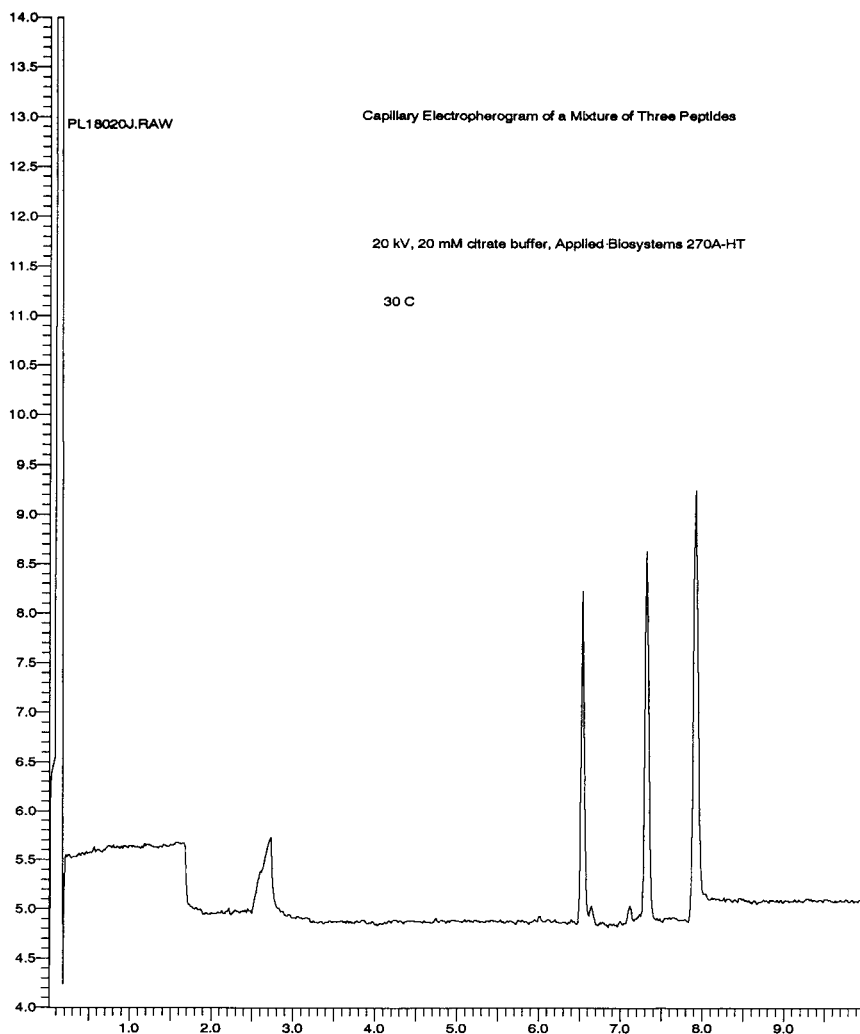


FIGURE 4.86 Capillary electropherogram for the separation and detection of three peptides from a Model 270A-HT[®] (Applied Biosystems).

potassium salts of the common inorganic anions were introduced by hydrodynamic injection into the Model 270A-HT. A potential of -25 kV with a thermostatically controlled temperature of 30°C in a 25 mM phosphate buffer were the CE conditions used. The order of ion migration is shown in Fig. 4.87 and differs from the ion chromatographic elution order dis-

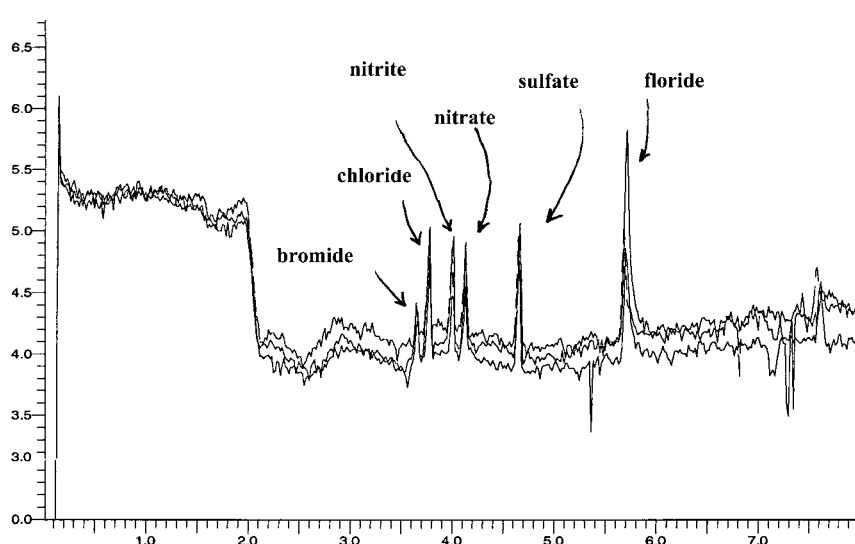
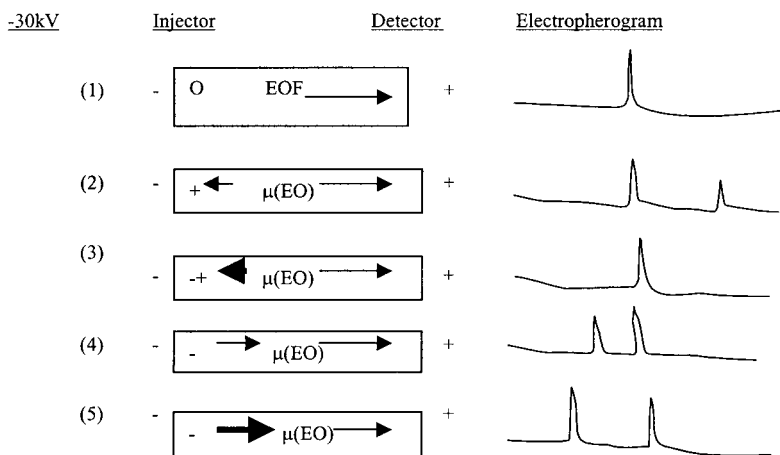


FIGURE 4.87 Capillary electropherogram for the separation of inorganic anions using indirect photometric detection: overlay of replicate injections of a 10-ppm reference standard.

cussed earlier. The peaks are actually negative peaks because an UV-absorbing chromophore has been added to the buffer. The lead wires from the detector were reversed and then connected to the interface. The schematic for this scenario is as follows:



83. WHAT IS VACANCY OR INDIRECT UV ABSORPTION DETECTION?

This technique has been used in HPLC to detect those analytes that either lack an UV chromophore in their molecular structure or have such a weak molar absorptivity, ϵ . A strong UV-absorbing chromophore is added to the mobile-phase in HPLC and to the buffer in CE (123). For the separation, detection, and quantification of the common inorganic anions, vacancy or indirect UV photometric detection (IPD) is the only means that exist to quantitate all of the common anions! The detector wavelength is set usually at that λ where a maximum in the absorption spectrum for the chromophore is located. A research group at Waters Associates has made significant progress in method development for CE-IPD (124). The methodology developed by this group has been recently applied in this author's laboratory to adapt the CE-IPD determinative technique to environmental geochemical related problems (125). We close our discussion of CE by examining the influence of buffer pH on CE migration time and migration order.

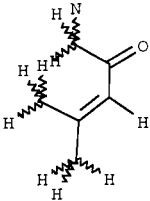
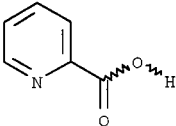
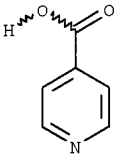
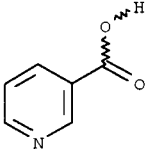
84. HOW IMPORTANT IS BUFFER pH IN CE?

“Quite important” is the answer; however, it depends on the secondary equilibrium characteristics of the analyte of interest. Consider the three isomeric pyridinium carboxylates listed in Table 4.23 along with a neutral marker, mesityl oxide. The electropherograms were developed using a 25 mM phosphate buffer and an applied potential of +25 kV. A plot of the migration time versus the buffer pH is shown in Fig. 4.88. Note that the migration time for neutral marker is not influenced by pH in contrast to the migration times for all three isomeric pyridinium carboxylates. PA, INA, and NA all exhibit a faster migration time relative to the migration time of MO at pH 2. A decrease in their migration times at increasing pH becomes evident as the pH reaches the “crossover pH” at around 3, as indicated in the plot. The order of migration actually reverses itself after this crossover pH has been passed as indicated in Fig. 4.88!

85. WHAT IS IN STORE FOR DETERMINATIVE TECHNIQUES FOR TEQA DOWN THE ROAD?

Chromatographic and atomic spectral determinative techniques are mature and one should expect few, if any, breakthroughs. Priority pollutant contaminants, both organic and inorganic, are quantitated down to very low concentration levels in a variety of sample matrices. This author envisions the need to commercialize the hyphenated techniques as has been accom-

TABLE 4.23 Name, Structure, First and Second Acid Dissociation Constants for Compounds Discussed in Fig. 4.88

Analyte	Structure	pK_{a1}	pK_{a2}
<u>Analyte</u>	<u>Structure</u>	<u>pK_{a1}</u>	<u>pK_{a2}</u>
Mesityl oxide		---	----
PA		1.06	5.37
INA		1.70	4.89
NA		2.07	4.73

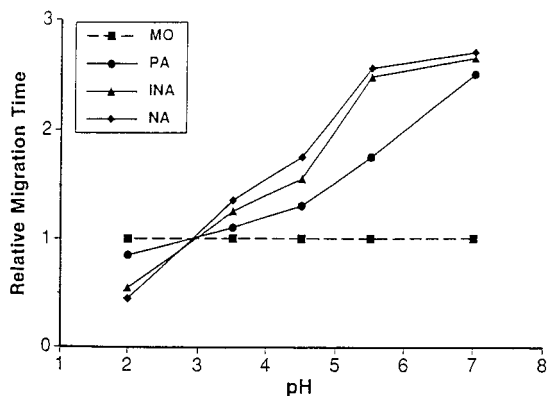


FIGURE 4.88 Plot of CE relative migration time versus buffer pH.

published for GC-MS and LC-MS. The interfacing of GC techniques with ICP-MS, HPLC techniques with ICP-MS, and in a manner similar to that recently discussed by Caruso who states the following (126):

Elemental speciation requires the coupling of two techniques: a separation technique and a detection technique, which in this case is mass spectrometry. The main separation techniques in elemental speciation are HPLC, CE, GC, and SFC. Each of these speciation techniques has its own advantages and preferred uses, and each tends to have its own special requirements with respect to interfacing with plasma MS detection . . . Time resolved data analysis is essential for any multi-element chromatographic or electrophoretic separation . . . rapid data acquisition can be performed using ICP-MS instruments equipped with software capable of monitoring signal versus time at several different mass-to-charge-ratios . . . growth in this technological area will greatly improve the use of speciation techniques.

This author wonders what our fictitious and uninformed observer now thinks about determinative techniques in TEQA!

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5

Specific Laboratory Experiments

This chapter provides a series of laboratory experiments that attempt to show some examples of how to conduct trace environmental quantitative analysis (TEQA) in light of what has been discussed in Chapters 2–4. I. M. Kolthoff, late Professor of Analytical Chemistry at the University of Minnesota, is credited with the saying, “Theory Guides, Experiment Decides.” It is in this chapter that the reader will acquire some of the flavor of experimental TEQA. These experiments were written by the author before the first four chapters were created. The impetus for writing these experiments was in support of a graduate-level course titled “Environmental Analytical Chemistry Laboratory.” This course began in the mid-90s and the instruction followed the installation of a teaching analytical laboratory coordinated by the author at Michigan State University in the Department of Civil and Environmental Engineering. The idea that evolved was to provide a set of learning exercises that introduces a student to the following thrusts:

- Review of safety guidelines related to work in a hazardous waste analysis lab
- Measuring the pH of snow and an artificially contaminated soil sample

- Correlating the presence of inorganic anions with pH via ion chromatography
- Introducing visible spectrophotometry and quantitatively determine ferric from ferrous ion in groundwater
- Introducing liquid–liquid extraction (LLE) and relating this sample prep technique to a visible spectrophotometer determinative technique while quantitatively determining a common anionic surfactant in wastewater
- Introducing molecular ultraviolet absorption spectrophotometry and showing how this relates to molecular structure
- Introducing molecular infrared absorption spectrophotometry and showing how this relates to molecular structure
- Introducing both flame and graphite furnace–atomic absorption spectrophotometry and showing how these determinative techniques relate to TEQA
- Introducing static headspace capillary GC and showing how this combined sample prep/determinative technique is used to evaluate the contamination of groundwater by gasoline
- Introducing soil extraction/leaching and showing how high-performance liquid chromatography (HPLC) is used to isolate and quantify a contaminated soil with polycyclic aromatic hydrocarbons (PAHs).

There are several options that an instructor can use to design a laboratory program that gives students the opportunity to measure environmentally significant chemical analytes. It is this author's opinion that it does not really matter which analytes are to be quantitated as long as an appropriate mix of sample prep and instrumental techniques are applied. One laboratory schedule that was used during the 1995–96 academic year is now considered.

1. WHAT MIGHT A TYPICAL LABORATORY SCHEDULE LOOK LIKE?

Listed below is the laboratory program implemented by the author for a course in TEQA. Under each experiment title is a statement about what outcomes the student will realize. The degree to which the instructor makes the course more or less rigorous is determined by the curriculum objectives. The degree of rigor can be determined by the instructor and the course objectives. An experimental course in TEQA can consist of a series of experiments with everything set up for the student at the less rigorous level or consist of the same experiments whereby the student does every-

thing. Some compromise between these two extremes might be the most appropriate.

A series of actual student experiments given as individual handouts follows this laboratory course outline.

<i>Project No.</i>	<i>Description</i>
First Seven Weeks	Orientation to Laboratory Discussion of Outcomes and What's Expected Definition of and Assignment to Workstations Safety Requirements Waste Disposable Regulations (ORCBS) <i>Descriptive introductory information</i>
1	Introduction to Visible Spectrophotometry and Determination of Fe(III)/Fe(II) in Groundwater or Determination of PO_4^{3-} in Surface Waters <i>Quantitative Analysis; emphasis on standards preparation techniques; statistical treatment of data; environmental sampling techniques; learning to operate the UV-vis spectrophotometer; learning to operate the flame atomic absorption (AA) spectrophotometer; no write-up required</i>
2	Determination of Anionic Surfactants by Micro-Liquid-Liquid Extraction (μLLE) Using Ion-Pairing with Methylene Blue <i>Quantitative Analysis; emphasis on sample preparation, unknown sample analysis; write-up required</i>
3	Ultraviolet Absorption Spectroscopy or Infrared Absorption Spectroscopy or Fluorescence Spectroscopy* <i>Qualitative Analysis; introduction to molecular spectroscopic instrumentation; sampling techniques; write-up required</i>
4	Determination of the Degree of Hardness in Groundwater Using Flame Atomic Absorption Spectroscopy: Measuring Ca, Mg, and Fe <i>Quantitative Analysis; calibration using external standard mode; spiked recovery; no write-up required</i>

- 5 **Determination of Lead in Drinking Water Using Graphite Furnace Atomic Absorption Spectroscopy**
Quantitative Analysis; learning to use the WinLab software for furnace atomic absorption spectroscopy; calibration based on standard addition; no write-up required

- 6 **Comparison of Soil Types via a Quantitative Determination of the Chromium Content Using Visible Spectrophotometry and Flame Atomic Absorption Spectroscopy**
Quantitative Analysis; use of two instrumental methods to determine the Cr(III) and Cr(VI) oxidation states; digestion techniques applied to soils; write-up required

Second Seven Weeks

- 7 **An Introduction to Data Acquisition and Control Using Turbochrom and an Introduction to High-Performance Liquid Chromatograph (HPLC): Evaluating Those Experimental Parameters Which Influence Instrument Performance**
Qualitative Analysis; emphasis on learning to operate the HPLC and the Turbochrom software; no write-up required; answer questions in lab notebook

- 8 **Identifying the Ubiquitous Phthalate Esters in the Environment Using HPLC, Photodiode-Array Detection (PDA), and Possible Confirmation by Gas Chromatography–Mass Spectrometry (GC–MS)**
Qualitative Analysis; interpretation of chromatograms, UV–absorption spectra, mass spectra; experience with GC–MS; write-up required

- 9 **An Introduction to Gas Chromatography: Evaluating Experimental Parameters Which Affect Gas Chromatographic Performance**
Qualitative Analysis; emphasis on learning to operate the GC; measurement of split ratio; no write-up required; answer questions in lab notebook

- 10 **Determination of Priority Pollutant Volatile Organic Compounds (VOCs) in Wastewater: Comparison of Sample Preparation Methods— μ LLE versus Statis Headspace Sampling**
Quantitative Analysis; unknown sample analysis; statistical treatment of data; write-up required
- 11 **Determination of the Herbicide Residue Trifluralin in Soil from Lawn Treatment by Gas Chromatography Using Solid-Phase Extraction (SPE) Methods**
Quantitative Analysis; calibration based on internal standard mode; unknown sample analysis; statistical treatment of data; write-up required
- 12 **Determination of Priority Pollutant Non-Volatile Organochlorine Pesticides in Contaminated Groundwater: Comparison of Sample Preparation Methods— μ LLE versus Solid-Phase Extraction Techniques***
Quantitative Analysis; emphasis on sample preparation, unknown sample analysis; calibration based on internal mode; statistical treatment of data; write-up required
- 13 **Determination of Selected Priority Pollutant Polycyclic Aromatic Hydrocarbons in Oil Contaminated Soil using LLE–RP–HPLC–PDA/ Determination of Oil and Grease in Contaminated Soil via Quantitative Fourier-Transform Infrared Spectrophotometry***
Quantitative Analysis; sample preparation; write-up required

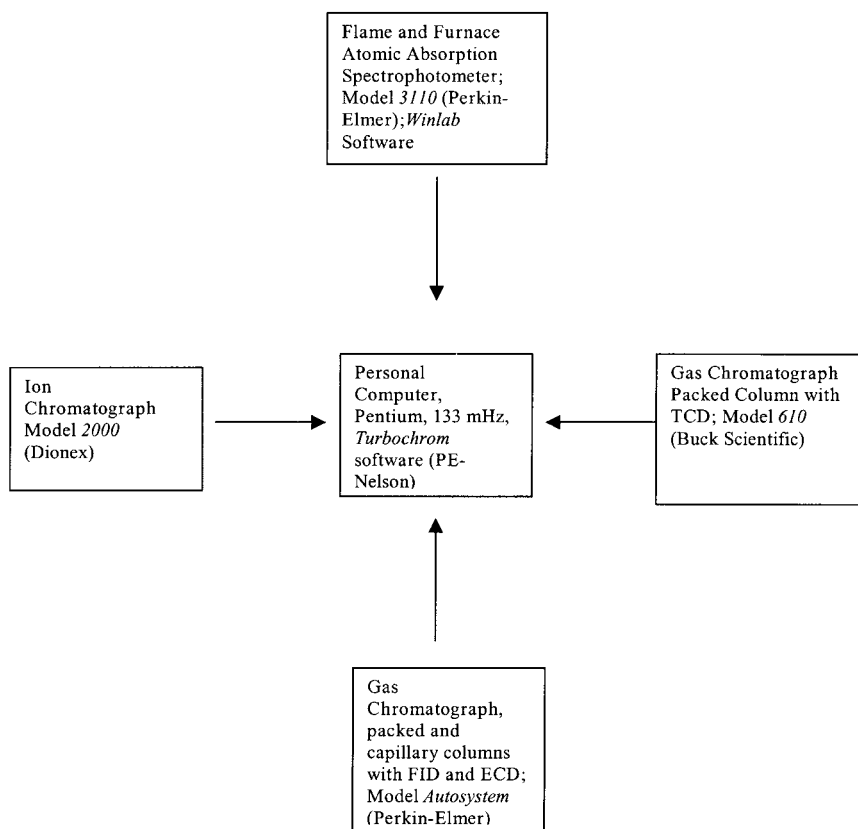
* Projects are considered *extra credit* and thus not required. Students must make arrangements with the laboratory instructor in order to perform these experiments.

This is a very ambitious one-semester laboratory schedule. To effectively educate students while delivering the course content requires a dedicated support staff, a committed faculty, sufficient laboratory glassware and accessories, and expensive analytical instrumentation, including interface of each instrument to a PC that operates chromatography or spectroscopy software. Each lab session requires at a minimum, four hours and at a

maximum, 8 hours. Students must be taught not only how to prepare environmental samples for trace analysis but also how to operate sophisticated analytical instrumentation. The intensity of the lab activities starts from an initial and less rigorous laboratory session toward increasing rigor as each session unfolds.

2. HOW IS THE INSTRUCTIONAL LABORATORY CONFIGURED?

The following is a schematic drawing of one of the four workstations that were installed in the Hazardous Waste Analysis Laboratory in the Department of Civil and Environmental Engineering at Michigan State University:

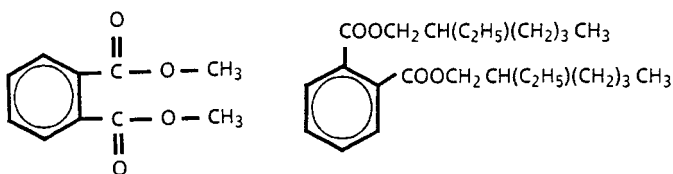


**IDENTIFYING THE UBIQUITOUS PHTHALATE ESTERS IN THE ENVIRONMENT USING
HPLC, PHOTODIODE-ARRAY DETECTION, AND CONFIRMATION BY GC-MS**

Background and Summary of Method

The most commonly found organic contaminant in landfills and hazardous waste sites has proven to be the homologous series of aliphatic esters of phthalic acid. This author has found phthalate esters in almost every Superfund waste site sample that he personally analyzed during the period 1986–90 while employed in an environmental testing laboratory in New York.

The molecular structures for two representative phthalate esters are drawn below (1). Dimethylphthalate (DMP) and bis(2-ethylhexyl)phthalate (bis) represent examples of a lower-molecular-weight phthalate ester to a higher-molecular-weight ester. DMP and the higher homologs, diethyl (DEP), di-*n*-propyl (DPP) and di-*n*-butyl phthalates (DBP), are the focus of this exercise.



The photodiode-array UV absorption detector provides both spectral peak matching and, if desired, peak purity determinations. This is nicely illustrated in Figures 5.1 and 5.2 (2). In Figure 5.1, the UV absorption spectrum from the peak at or near 39 min in the HPLC chromatogram is retrieved from a stored library file. The UV spectrum for the peak and that for a reference standard are compared.

Figure 5.2 overlays UV absorption spectra for three points along the Gaussian chromatographically resolved peak and uses an algorithm to calculate a purity match. Note the difference between the overlaid UV absorption spectra for the impure versus the pure peak. You will not be using the peak purity algorithm in this exercise.

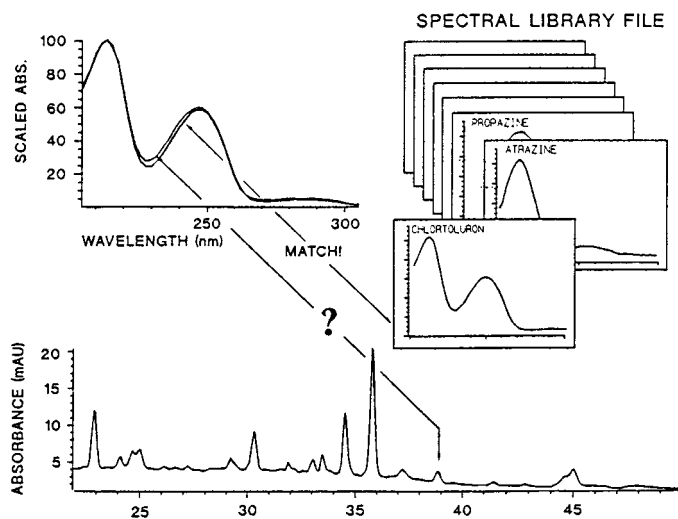


FIGURE 5.1 Spectral peak matching.

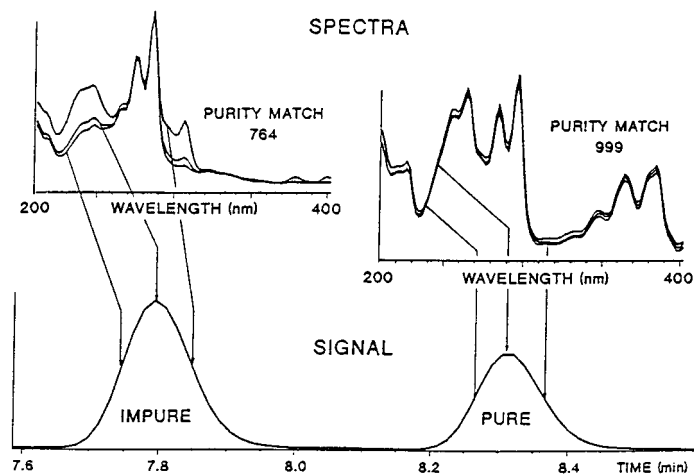


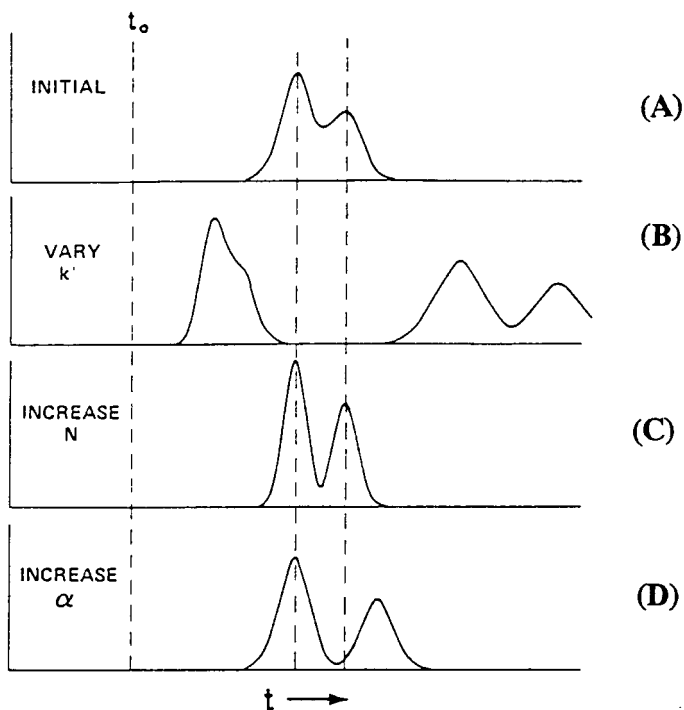
FIGURE 5.2 Peak purity determination by spectral overlay.

Analytical Method Development Using HPLC

Analytical method development in HPLC usually involves changing the composition of the mobile phase until the desired degree of separation of the targeted organic compounds has been achieved. One starts with a mobile phase which has a high “solvent strength” and moves downward in solvent strength to where a satisfactory resolution can be achieved. Recall the key relationship for resolution (3):

$$R_S = \frac{1}{4}(\alpha - 1)(N)^{1/2} \left(\frac{k'}{1 + k'} \right)$$

A useful illustration of the effects of selectivity, plate count, and capacity factor follows:



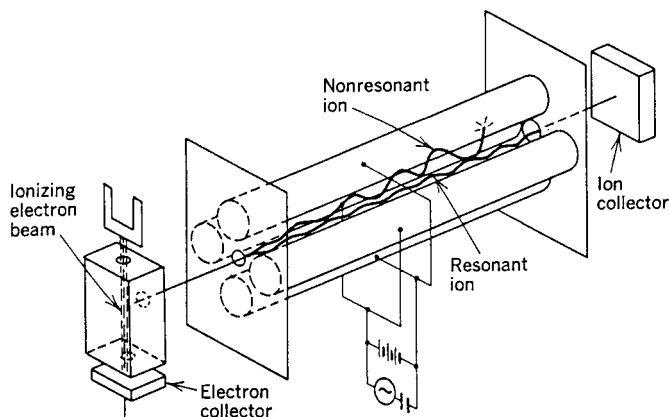
HPLC chromatogram (A) shows a partial separation of two organic compounds, e.g., DMP from DEP. This degree of resolution, R_S could be improved by changing k' , N , or α . In (B), k' is increased, which changes the retention times and shows a slight improvement in R_S . Increasing N

significantly increases R_S , as is shown in (C); the greatest increase in R_S is obtained by increasing α , as is shown in (D). Refer to your text or an appropriate monograph on HPLC to enlarge on these concepts.

GC-MS Using a Quadrupole Mass Spectrometer

In a manner similar to obtaining specific UV absorption spectra for chromatographically separated peaks as in HPLC-PDA, GC-MS also provides important identification of organic compounds first separated by gas chromatography. The mass spectrometer that you will use consists of four rods arranged to form parallel sides of a rectangle as shown below. The beam from the ion source is directed through the quadrupole section as shown below.

The quadrupole rods are excited with a large dc voltage superimposed on a radio frequency (RF) voltage. This creates a three-dimensional, time-varying field in the quadrupole. An ion traveling through this field follows an oscillatory path. By controlling the ratio of RF to DC voltage, ions are selected according to their mass-to-charge ratio. Continuously sweeping the RF/DC ratio will bring different m/z ratios across the detector. The GC-MS available at the Research Complex combines a 5890 GC (Hewlett-Packard) with an Incos 50B quadrupole mass spectrometer (Finnigan). Both the GC and MS are controlled via a DG-10 microcomputer (Data General). The following is a schematic of the MS (4):



Of What Value Is This Experiment?

The goal of this experiment is to provide an opportunity for students to engage in analytical method development by **identifying an unknown phtha-**

late ester provided to them. This is an example of **qualitative analysis**. The reference standard solution consists of a mixture of the four phthalate esters, DMP, DEP, DPP, and DBP. Each group will be given an unknown which contains one or more of these phthalate esters. A major objective would be to use available instrumentation to achieve the goal. Students will have available to them an HPLC in the reversed-phase mode (RP-HPLC) and access to the department's gas chromatograph-mass spectrometer system, which is located in the Research Complex-Engineering.

Students must first optimize the separation of the esters using RP-HPLC, record and store the ultraviolet absorption spectra of the separated esters, and compare the spectrum of the unknown against the stored UV spectra. In addition, staff will be available to conduct the necessary GC-MS determination of the unknown. A hardcopy of the chromatogram and mass spectrum will be provided so that the student will have additional confirmatory data from which to make a successful identification of the unknown phthalate ester.

Experimental

High-performance liquid chromatograph set up for reversed-phase separations

Capillary gas chromatograph-mass spectrometer incorporating a quadrupole mass selective detector or ion trap mass selective detector

Preparation of Chemical Reagents

NOTE: ALL REAGENTS USED IN THIS ANALYTICAL METHOD CONTAIN HAZARDOUS CHEMICALS! WEAR APPROPRIATE EYE PROTECTION, GLOVES, AND PROTECTIVE ATTIRE. USE OF CONCENTRATED ACIDS AND BASES SHOULD BE DONE IN THE FUME HOOD.

Accessories to Be Used with the HPLC per Student or Group

- 1 HPLC syringe (this syringe incorporates a blunt end; use of a beveled-end GC syringe would damage inner seals to the Rheodyne HPLC injector)
- 1 Four-component phthalate ester standard; check the label for concentration values
- 1 Unknown sample which contains one or more phthalate esters; be sure to record the code for the unknown assigned

Procedure

Unlike previous exercises, **no methods have been developed for this exercise**. Consult with your lab instructor as to the details for developing a general strategy. You will be introduced to Turboscan, which is the name of the software which will allow you to store and retrieve UV absorption spectra.

First, find the mobile phase solvent strength which optimizes the separation of the four phthalate esters. **Second**, retrieve the UV absorption spectrum for each of the four and build a library. **Third**, inject the unknown sample and retrieve its UV spectrum. **Fourth**, make arrangements with the staff to get your unknown sample analyzed using GC–MS which is available at the Research Complex–Engineering.

For the Report

Include your unknown phthalate ester identification code along with the necessary laboratory data and interpretation of results to support your conclusions.

Please address the following (include these in your report):

1. Compare the similarities and differences for the homologous series of phthalate esters on both UV absorption spectra and on mass spectra from your data.
2. Explain what you would have to do if you achieved the optimum resolution and suddenly ran out of acetonitrile! Assume that you have only methanol available in the lab. Would you use the same mobile-phase composition in this case?
3. This exercise introduces you to the quadrupole mass filter. Briefly describe how the mass spectrum is obtained and if you so desire, attempt to provide a brief mass spectral interpretation. You may want to review a text that introduces GC-MS, such as that cited in Ref. 5.

References

1. Product catalog, Research Triangle Park, NC: NSI Environmental Solutions, 1991.
2. George S. In: Huber, George, eds. Diode-Array Detection in HPLC. Marcel Dekker, Inc., 1993, pp 48–49.
3. Snyder L, J Kirkland. Introduction to Modern Liquid Chromatography. New York: John Wiley & Sons, 1979, p 49.
4. Ewing G. Instrumental Methods of Chemical Analysis. 5th ed. New York: McGraw-Hill, 1985, p 409.

5. Skoog D, J Leary. Principles of Instrumental Analysis. 4th ed. Philadelphia: WB Saunders, 1992, pp 642–647.

**DETERMINATION OF PRIORITY POLLUTANT POLYCYCLIC AROMATIC HYDROCARBONS
(PAHs) IN CONTAMINATED SOIL USING RP-HPLC-PDA WITH WAVELENGTH
PROGRAMMING**

Background and Summary of Method

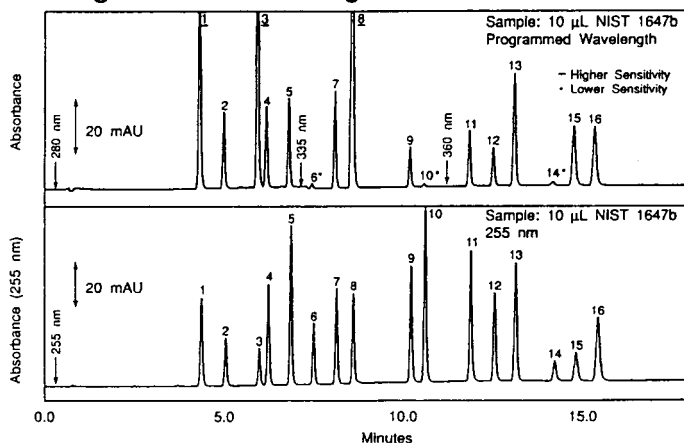
In 1979, the EPA proposed Method 610, which, if properly implemented, would determine the 16 priority pollutant PAHs in municipal and industrial discharges (1). The method was designed to be used to meet the monitoring requirements of the National Pollutant Discharge Elimination System (NPDES). The assumption used was that a high expectation of finding some, if not all, of the PAHs was likely. The method incorporated packed column GC in addition to HPLC, and because of the inherent limitation of packed columns, it was unable to resolve four pairs of compounds (e.g., anthracene from phenanthrene). Because RP-HPLC could separate all 16 PAHs, it became the method of choice. The method involved extracting a 1-L sample of wastewater using methylene chloride, use of Kuderna–Danish evaporative concentrators to reduce the volume of solvent, cleanup using a silica gel micro-column, and a solvent exchange to acetonitrile prior to an injection into an HPLC system. The method requires that a UV absorbance detector and a fluorescence detector be connected in series to the column outlet. This affords maximum detection sensitivity because for some PAHs (e.g., naphthalene, phenanthrene, fluoranthene, among others) are much more sensitive when detected by fluorescence than by UV absorption.

In most laboratories today, PAHs are routinely monitored under EPA Method 8270 and comprise the majority of “neutrals” under the Base, Neutral, Acids (BNAs) designation of the method (2). This is a liquid–liquid extraction method with determination by gas chromatography–mass spectrometry (GC–MS). Careful changes in pH of the aqueous phase enables a selective extraction of bases and neutrals from acidic compounds. Examples of **priority pollutant organic bases** include aniline and substituted anilines. Examples of **priority pollutant organic acids** include phenol and substituted phenols. The most popular method of recent years has been EPA Method 525, which incorporates SPE techniques and is applicable to PAHs in drinking water (3).

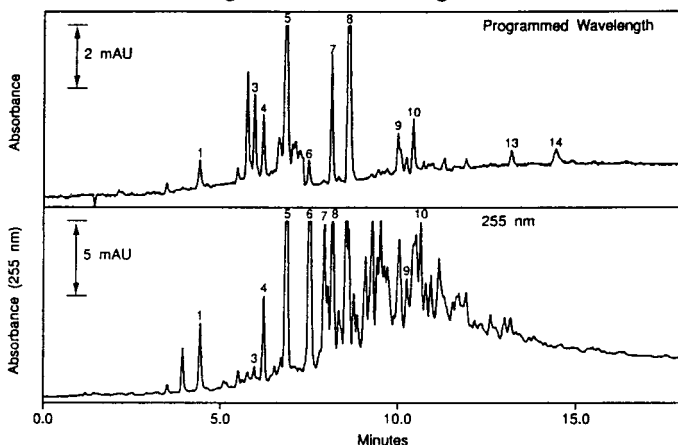
The most common wavelength, λ , for use with aromatic organic compounds is generally 254 nm because almost all molecules which incorporate the benzene ring in their structure absorb at this wavelength. This wavelength may or may not be the most sensitive wavelength for most PAHs.

The two figures below compare RP-HPLC chromatograms for the 16 priority pollutant PAHs in a reference standard mixture and from a soil extract. In the lower chromatogram of each figure, λ was held fixed at 255 nm, whereas for the upper chromatogram of each figure, λ was changed during the run so as to demonstrate how the wavelength influences peak height (4). The wavelength-programmed HPLC chromatogram shows much less background absorbance and, hence, increased sensitivity. This information should be used in developing the wavelength-programmed HPLC method.

Comparison of UV Detection at 255 nm with Programmed Wavelength



Soil Extract 255 nm vs Programmed Wavelength



Of What Value Is This Experiment?

This exercise affords the student an opportunity to build a new HPLC method using the chromatography data handling software. The method will also incorporate the concept of “wavelength programming,” whose objective is to maximize detector sensitivity for a given analyte and which can only be performed using a PDA detector and accompanying digital electronics. The following table summarizes the detection limits for λ 255, 280 and for UV programming during the chromatographic run:

Sensitivity and Linearity Data for UV Absorption Detection

No.	PAH	$\lambda =$		
		255 nm (ng)	280 nm (ng)	Programmed (ng)
1	Naphthalene	0.6	0.7	0.7 (280 nm)
2	Acenaphthylene	0.9	1.9	1.9
3	Acenaphthene	1.42	0.60	0.60
4	Fluorene	0.13	0.53	0.53
5	Phenanthrene	0.06	0.36	0.36
6	Anthracene	0.03	2	1.2 (335 nm)
7	Fluoranthene	0.22	0.24	0.45
8	Pyrene	0.25	1.1	0.07
9	Benz(<i>a</i>)anthracene	0.09	0.08	0.5
10	Chrysene	0.06	0.41	6.0
11	Benzo(<i>f</i>)fluoranthene	0.09	0.23	0.4 (360 nm)
12	Benzo(<i>k</i>)fluoranthene	0.14	0.31	0.6
13	Benzo(<i>a</i>)pyrene	0.11	0.2	0.2
14	Dibenz(<i>a,h</i>)anthracene	0.45	0.14	4
15	Benzo(<i>g,h,i</i>)perylene	0.32	0.32	0.3
16	Indeno(1,2,3, <i>c,d</i>)pyrene	0.16	0.38	0.35

Experimental

High Performance Liquid Chromatograph that incorporates a UV absorption detector under reversed-phase conditions

Preparation of Chemical Reagents

NOTE: ALL REAGENTS USED IN THIS ANALYTICAL METHOD CONTAIN HAZARDOUS CHEMICALS! WEAR APPROPRIATE EYE PROTECTION, GLOVES, AND

PROTECTIVE ATTIRE. USE OF CONCENTRATED ACIDS AND BASES SHOULD BE DONE IN THE FUME HOOD.

Accessories to Be Used with the HPLC per Group

- 1 HPLC syringe (this syringe incorporates a blunt end; use of a beveled-end GC syringe would damage inner seals to the Rheodyne injector)
- 1 Sixteen-component PAH standard; check the label for concentration values

Procedure

Be sure to record your observations in your laboratory notebook.

Creating the Wavelength Program Method

Again, you will first find the HPLC instrument in the off position; use “hands on” to activate the instrument and allow at least 15 min for the detector to warm up and stabilize. Ask your laboratory instructor for assistance if necessary. Observe the variability in baseline absorbance. Absorbance should not vary much above a ΔA of 0.0100. Significant variability is most often due to trapped air bubbles due to insufficient degassing of the mobile phase. Inform your instructor if this baseline absorbance variation is significant.

Once the baseline is stable, retrieve the method titled “PAH255” and download it. This method is one previously created by the instructional staff and is a fixed wavelength (λ @ 255 nm). Fill the 5- μ L injection loop with the PAH standard and observe the chromatogram. The method separates the PAHs based on gradient elution. The method incorporates a one-point calibration.

Use the above tabular information and modify this method to incorporate wavelength programming as discussed earlier. Save the modified method as “PAHWP” where WP stands for “wavelength programmed.” Ask your laboratory instructor for assistance in developing this software capability. Fill the 5- μ L injection loop with the PAH standard. Using the “chromatograms” section in the main menu, proceed to retrieve both HPLC chromatograms that you just generated. Use the overlay capability to compare both chromatograms and print the overlay. Update the one-point calibration with this standard. You should not have a new method with an updated calibration prior to injecting the extract from the soil discussed below.

Extraction Procedure for Soil

Weigh approximately 2.0 g of contaminated soil into a 50- or 125-mL glass beaker. Add 20 mL of methylene chloride and use a glass stirring rod to facilitate mixing. Let the contents of the mixture stand for at least 10 min. Decant the extract into a second beaker. It may be necessary to filter this extract if particulates become a problem. This will depend on the type of sample. Pipette 1.0 mL of the methylene chloride extract into a clean, dry 10-mL volumetric flask. Adjust to the calibration mark with acetonitrile. Fill the injection loop with this diluted extract. It may be necessary to use a 0.45- μm syringe filter to remove particulates from the diluted extract. Fill the HPLC syringe with about five times the loop volume to assure a reproducible injection volume. The peak area that is found refers to the concentration of a given PAH in the diluted extract. You will be given assistance on how to allow Turbochrom to calculate the concentration of each PAH in the original contaminated soil. If time permits, make a second injection of the diluted extract. **Discard the excess methylene chloride extract and CH_2Cl_2 /ACN diluted extract into the ORCBS waste receptacle when finished.**

Calculation of the ppm of Each PAH in Contaminated Soil

Let us assume that upon injection of the diluted soil extract, a concentration of 225 ppm of dibenzo(*a,h*)anthracene in the diluted soil extract was obtained based on a correctly calibrated instrument.

What would the original concentration of dibenzo(*a,h*)anthracene be in the contaminated soil?

225 ppm means 225 $\mu\text{g/mL}$ of diluted soil extract

Thus, $225 \times 10 = 2250 \mu\text{g/mL}$ in the original 20 mL extract before dilution

One says that the dilution factor “DF” is 10

(20 mL extract)[2250 $\mu\text{g/mL}$ dibenzo(*a,h*)anthracene] = 45,000 μg total from 2 g soil

45,000 μg total/2.0 g soil = 22,500 $\mu\text{g/g}$ or ppm dibenzo(*a,h*)anthracene in contaminated soil

Upon properly completing the sequence file within Turbochrom, the final result, 22,500 ppm, will be directly obtained in the “Peak Report” for that sample.

For the Report

Include the overlay comparison and calibration results and list the concentration of each PAH in the contaminated soil sample. If a second sample

result is available, estimate the precision of the method. Comment on the advantage of using a PDA to increase sensitivity.

Address the following:

1. Explain the elution order for the 16 PAHs using chemical principles.
2. The method detection limit using an UV absorption detector for some of the 16 priority pollutant PAHs could be improved if a different detector could be used. Explain.
3. Explain why this method is considered “quick.” Are there limitations to the use of “quick” methods and, if so, what are some of these?

Some representative PAHs are as follows:

Compound	Abbreviation	M_r	Molecular formula	Molecular structure	Aqueous solubility	$\log(K_{ow})$
Naphthalene	NA	128	$C_{10}H_8$		31.7	3.36
Acenaphthylene	ACY	152	$C_{12}H_8$		16.1	3.94
Acenaphthene	ACE	154	$C_{12}H_{10}$		3.93	4.03
Fluorene	FLE	166	$C_{13}H_{10}$		1.98	4.47
Phenanthrene	PH	178	$C_{14}H_{10}$		1.29	4.57
Anthracene	AN	178	$C_{14}H_{10}$		0.073	4.54
Fluoranthene	FLA	202	$C_{16}H_{10}$		0.260	5.22
Pyrene	PY	202	$C_{16}H_{10}$		0.135	5.18
Triphenylene	TRP	228	$C_{18}H_{12}$		0.043	5.45
Benz(a)anthracene	BaA	228	$C_{18}H_{12}$		0.014	5.91
Chrysene	CHR	228	$C_{18}H_{12}$		0.002	5.91

References

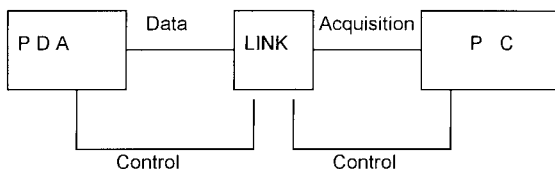
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2. Gas Chromatography–Mass Spectrometry for Semivolatile Organics: Capillary Column Technique, Method 8270. In: Test Methods for Evaluating Solid Wastes, SW 846, 3rd ed. Washington, DC: Office of Solid Waste, EPA, 1986.

3. Determination of Organic Compounds in Drinking Water by Liquid-Solid Extraction and Capillary Gas Chromatography/Mass Spectrometry. In: Methods for the Determination of Organic Compounds in Drinking Water, Method 525, EPA/600/4-88/039, Cincinnati, OH: EMSL, 1988.
4. Dong M, et al. A quick-turnaround HPLC method for PAHs in soil, water and wastewater oil. LC-GC 11(11): 802-810, 1993; also Quick Turnaround HPLC Method for PAHs in Soil, Water and Air Samples, courtesy of Perkin-Elmer Corporation.

**AN INTRODUCTION TO DATA ACQUISITION AND CONTROL USING TURBOCHROM AND
AN INTRODUCTION TO HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC):
EVALUATING THOSE EXPERIMENTAL PARAMETERS THAT INFLUENCE SEPARATIONS**

Background and Summary of Method

Contemporary analytical instrumentation is said to be “interfaced to computers.” These developments commenced in the early to mid-80s and took hold with Windows-based software environments in the 1990s. This can be illustrated as follows:



Interfaces can be either stand-alone or installed into the console of the PC. Instruments can be controlled and data acquired from a PC, or if control is not available, only data acquisition is obtained. In our laboratory, both types of interfaces are used. With appropriate software, the control and data acquisition tasks are easily performed. If a means can be acquired to enable automatic sampling to be controlled as well, a totally automated system results! This was accomplished in our laboratory.

The HPLC within each workstation is both PC controlled and the photodiode-array detector (PDA) is interfaced to the same PC, thus enabling real-time data acquisition. Students are first asked to study the present architecture so as to gain an appreciation of contemporary HPLC-PDA-DS (data system) technology.

This experiment is designed to take you through an initial “hands on” experience with the HPLC-PDA-DS from a first sample injection to a simple quantitative analysis. A “quick method” is first necessary for the system to recognize something. This is followed by optimizing the initial

method, conducting a calibration, creating a customized report format and evaluating the initial calibration verification (ICV).

Following completion of the initial experiment, the focus shifts to the separation of the test mixture or organic compounds using the HPLC instrument. The effect of solvent strength on k' and the effect of mobile-phase flow rate on R_S will be considered by retrieving previously developed Turbochrom methods and making manual injections.

HPLC and Trace Environmental Analysis

High-performance liquid chromatography followed GC in the early development of instrumental column chromatographic techniques that could be applied to trace environmental analysis. HPLC most always complements and sometimes duplicates GC. For example, polycyclic aromatic hydrocarbons (PAHs) can be separated and quantitated by both techniques; however, *N*-methylcarbamate pesticides can be determined by HPLC only as a result of the thermal instability in a GC injection port. HPLC has become the dominant instrumental analysis method for the pharmaceutical industry, yet continues to take a secondary role in the environmental field. Samples that contain the more polar and thermally labile analytes are much more amenable to analysis by HPLC rather than by GC. A major contaminant in a lake in California went undetected until State Department of Health chemists identified a sulfonated anionic surfactant as the chief cause of the pollution. This pollutant was found using HPLC techniques. HPLC encompasses a much broader range of applicability in terms of solute polarity and molecular-weight range when compared against GC. This is nicely illustrated in Ref. 1.

To illustrate how these different kinds of HPLC might aid the analyst in the environmental testing laboratory, consider the request from an engineering firm that wishes to evaluate the degree of phthalate ester contamination from leachate emanating from a hazardous waste site. Reversed-phase HPLC is an appropriate choice for the separation of lower-molecular-weight phthalate esters (e.g., dimethyl from diethyl from dibutyl). Attempts to elute higher-molecular-weight and much more hydrophobic (lipophilic) phthalate esters [e.g., dioctyl, bis(2-ethylhexyl) under reversed-phase conditions are unsuccessful!] The separation of these under normal-phase HPLC conditions is successful.

Flow-Through Packed Columns

High-performance liquid chromatography requires that liquid be pumped across a packed bed within a tubular configuration. Snyder and Kirkland have used the Hagen–Poiseuille equation for laminar flow through tubes

and Darcy's law for fluid flow through packed beds and derived the following relationship (2):

$$t_0 = \frac{15,000L^2\eta}{\Delta P d_p^2 f} \quad (1)$$

where t_0 is the retention time of an unretained solute (the time it takes after injection for an unretained solute to pass through the column and reach the detector), L is the length of the column, η is the viscosity of the mobile phase, ΔP is the pressure drop across the column, d_p is the particle size of the stationary-phase packing, and f is an integer and is 1 for irregular porous, 2 for spherical porous, and 4 for pellicular packings.

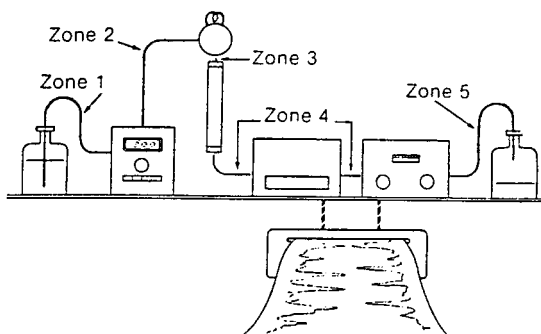
The importance of stationary-phase particle size is reflected in the dependence of the void retention volume $V_0 = F(t_0)$, where F is the mobile-phase flow rate in, for example, cm^3/min , on the inverse square of d_p . Recall that the retention volume of a retained solute whose capacity factor is given by k' is

$$V_R = V_0(1 + k') \quad (2)$$

Hence, the smaller the d_p , the larger is V_0 , and, consequently, V_R . A smaller d_p also contributes in a significant manner to a larger N (refer to theoretical equations found in Chapter 4).

High-Pressure Liquid Chromatograph

It is quite useful to view the instrumentation for HPLC in terms of zones according to the following schematic (3):



Zone 1—low-pressure zone prior to pump. This is a noncritical area served by Teflon tubing. A fritted filter is placed at the inlet to prevent particulates from entering the column.

Zone 2—high-pressure zone between pump and injector. This is a noncritical area served by standard stainless-steel (SS) tubing usually 1/16 in. in outer diameter (O.D.). A high-surface-area 0.5- μm filter can be placed here to prevent particulates from reaching the column.

Zone 3—high-pressure area surrounding injector and column. This is a critical area where the sample is introduced to the separation system. The volume must be well swept and minimized. Special fittings are 0.25-mm-inner diameter (I.D.) SS tubing.

Zone 4—low-pressure area between column and detector. In this critical area, separation achieved in the column can be lost prior to detection. The volume must be well swept and minimized. Special fittings and 0.25-mm SS or plastic tubing are required. The critical zone extends to all detectors or fraction collectors in series or parallel connection.

Zone 5—low-pressure area leading to waste collector. This noncritical area is served by Teflon tubing. Most labs fail to fit the waste vessel with a vent line to the hood or exhaust area.

Experimental

High-performance liquid chromatograph incorporating an UV absorption detector under reversed-phase conditions

Preparation of Chemical Reagents

NOTE: ALL REAGENTS USED IN THIS ANALYTICAL METHOD CONTAIN HAZARDOUS CHEMICALS! WEAR APPROPRIATE EYE PROTECTION, GLOVES, AND PROTECTIVE ATTIRE. USE OF CONCENTRATED ACIDS AND BASES SHOULD BE DONE IN THE FUME HOOD.

Accessories to Be Used with the HPLC per Group

- 1 HPLC syringe (this syringe incorporates a blunt-end; use of a beveled-end GC syringe would damage inner seals to the Rheodyne injector).
- 1 10-mL two-component mix at 1000 ppm each; prepare the mixture by dissolving 10 mg of phthalic acid (PhtA) and 10 mg of dimethylphthalate (DMP) in about 5 mL of 50 : 50 ACN : H_2O

in a 50-mL beaker. After dissolution, transfer to a 10-mL volumetric flask and adjust to the final mark with the 50 : 50 solution.

Procedure

Be sure to record your observations in your laboratory notebook.

Initial Observations of a Computer-Controlled High-Performance Liquid Chromatograph

Upon approaching the HPLC–PDA–DS, conduct the following:

1. Identify each of the five zones discussed above.
2. Locate the following hardware components:
 - (a) The IEEE-488 cable to the LINK interface
 - (b) The start/stop line from the Rheodyne injector to the LINK
 - (c) The data acquisition line from the PDA to the LINK
 - (d) The Keyring and Master Key

Creating a QuickStart Method, Acquiring Data, Optimizing, Calibrating, and Conducting Analysis Using the QuickStart Method

Proceed with the “Turbochrom 4 Tutorial” and create a method using QuickStart. Inject a 100-ppm test mix reference standard. Optimize the method using the Graphic Editor. Develop the calibration and report format sections of your method. Establish a three-point calibration for DMP only between 10 and 100 ppm (inject from low concentration to high) and prepare an ICV run the ICV in triplicate.

Effect of Solvent Strength on k'

A good practice when beginning to use a RP–HPLC instrument is to initially pass a mobile phase which contains 100% acetonitrile (ACN) so as to flush out of the reversed-phase column any nonpolar residue that might have been retained from previously running the instrument. Retrieve the Turbo method titled “100% ACN” and download if not already set up for you. **Download within “setup” using the “method” approach.**

Retrieve the method from Turbochrom titled “80%ACN” and proceed to use “Setup in the method mode” to enable you to operate the HPLC with a mobile-phase composition of 80% ACN and 20% aqueous. The use of “Setup” is called “downloading” the method and sequence file so that data acquisition can begin. The aqueous mobile phase consists of 0.05% phosphoric acid dissolved in distilled, deionized water (DDI). Carefully fill the 5- μ L injection loop (the injector arm should be in the “load” position with the evaluation test mix with the HPLC syringe). Inject by moving the

injector arm from the “load” position to the “inject” position. **Observe the chromatogram that results. Note the retention times of the components in the mixture. Give all members in the group the opportunity to make this initial injection.**

Retrieve the method from Turbochrom titled “60%ACN,” download it, and then proceed to repeat the injection procedure discussed above. **Observe the chromatogram that results and note retention times.**

Retrieve the method from Turbochrom titled “40%ACN,” download it, and then proceed to repeat the injection discussed earlier. **Observe the chromatogram that results and note retention times.**

Retrieve the method from Turbochrom titled “20%ACN,” download it, and then proceed to repeat the injection procedure discussed earlier. **Observe the chromatogram that results and note retention times.**

Effect of Mobile-Phase Flow Rate on Resolution

The mobile-phase flow rate will be varied and its influence on chromatographic resolution will be evaluated.

Retrieve the method from Turbochrom titled “FlowHi,” download it, and then proceed to use “Setup” as you did during the variation of solvent strength experiments. Allow sufficient equilibration time at this elevated mobile-phase flow rate. Notice what happens to the column back-pressure when a high flow rate is in operation. Inject the test mix and **observe the chromatogram that results.**

Retrieve the method from Turbochrom titled “FlowLo,” download it, and then proceed to repeat the injection procedure discussed earlier. **Observe the chromatogram that results.**

For the Lab Notebook

Review Section 24D and most of the relevant sections of Chapter 26 of Ref. 1. Note the summary equations found in Table 24-5.

The following empirical relationship has been developed for RP-HPLC (4):

$$\log k' = \log k_W - S\Phi \quad (3)$$

where k' is the capacity factor for a retained peak, k_W is the capacity factor (extrapolated) k' for pure water, Φ is the volume fraction of the organic solvent in the mobile phase, and S is a constant which is approximately proportional to solute molecular size or surface area.

Choose one component in the evaluation test mix and determine whether Eq. (3) is consistent with your observations.

Address the following:

1. Among the three major parameters upon which resolution R_S depends, which of the three is influenced by changes in mobile-phase flow rate? Explain.
2. Mr. Everett Efficient believes that he can conserve resources by operating his HPLC using a mobile phase which consists only of a 0.01M aqueous solution containing sodium dihydrogen phosphate. Discuss what is seriously deficient in Mr. Efficient's fundamental assumption.
3. Assume that you could change HPLC columns in this exercise and that you installed a column which contained 3- μ m particle size silica. Assume that you used the same mobile-phase composition that you used for the reversed-phase separations that you observed. Explain what you would expect to find if the reversed-phase test mix were injected into this HPLC configuration.
4. Explain why DMP is retained longer (i.e., has the higher k') than phthalic acid given the same mobile-phase composition.

References

1. Skoog D, J Leary. Principles of Instrumental Analysis. 4th ed. Philadelphia: WB Saunders, 1992, Fig. 26-1, p 629.
2. Snyder L, J Kirkland. Introduction to Modern Liquid Chromatography. 2nd ed. John Wiley & Sons, 1979, pp 36-37.
3. Guide to LC. Rainin Instruments Corporation.
4. Ahuja S. Selectivity and Detectability in HPLC. Chemical Analysis Series of Monographs on Analytical Chemistry and Its Applications, Volume 104. New York: Wiley-Interscience, 1989, p 28.

DETERMINATION OF PRIORITY POLLUTANT NON-VOLATILE ORGANOCHLORINE PESTICIDES: A COMPARISON OF MICRO-LIQUID-LIQUID AND SOLID-PHASE EXTRACTION TECHNIQUES

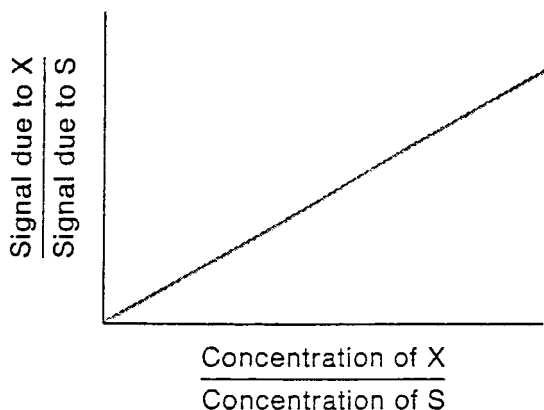
Background and Summary of Method

Organochlorine pesticides (OCs) were used widely in agriculture during the first half of the twentieth century in the United States and were subsequently banned from use during the 1970s. Unfortunately, some of the OCs are still in widespread use around the world. Their persistence in the environment was not apparent until Lovelock introduced the electron-capture detector (ECD) in 1960 (1). When combined with high-resolution capillary gas chromatography and appropriate sample preparation methods, the ECD provides the analytical chemist with the most sensitive means by which to

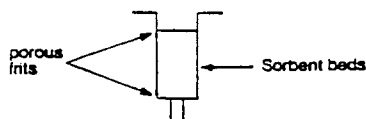
identify and quantitate OCs in environmental aqueous and soil/sediment samples. As analytical chemists were seeking to identify and quantitate OCs during the early 1970s, it became apparent that many additional chromatographically resolved peaks were appearing. What were considered as unknown interfering peaks in the chromatogram then were subsequently found to be polychlorinated biphenyls (PCBs).

The OCs and PCBs were first determined in wastewaters using EPA Method 608 (2). This method originally required packed columns, and because of this, it necessitated extensive sample preparation and cleanup techniques which included liquid–liquid extraction and low-pressure column liquid chromatography. Capillary GC–ECD when combined with more contemporary methods of sample preparation provides for rapid and cost-effective trace environmental analysis. Over the past 10 years, there has been dramatic improvements in sample preparation techniques as this relates to semivolatile and nonvolatile trace analyses.

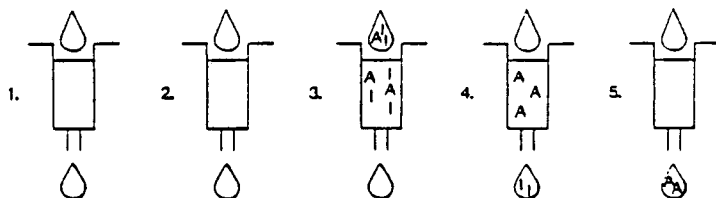
In addition to external standard and standard addition, the last principal mode of calibration is called internal standard. This mode of calibration should be used when there exists variability in sample injection volume, when there is concern about the lack of instrument stability, and when there is unavoidable sample loss. Instrumental response then becomes related to the ratio of the unknown analyte X to that for the internal standard S instead of related only to the unknown analyte. If some X is lost, one can assume that some S would be lost as well. This preserves the ratio $[X]/[S]$. For extraction methods, the internal standard (IS) is added to the final extract just prior to adjusting the final volume. Selecting a suitable IS is not trivial. It should possess similar physical and chemical properties to the analyte of interest and not interfere with the elution of any of the analytes that need to be identified and quantitated. The IS should be within the same concentration range as for the calibration standards and at a fixed concentration. The following is a calibration curve for the IS mode:



This exercise introduces the student to solid-phase extraction (SPE) techniques. The μ LLE method will also be implemented. The two methods will be compared. SPE in the reversed-phase (RP) mode of operation involves passing an aqueous sample over a previously conditioned sorbent which contains a chemically bonded silica gel held in place with polyethylene frits within a column configuration. A typical RP-SPE sequence follows:

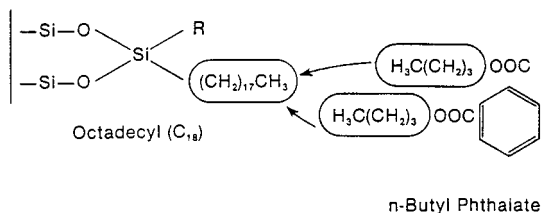


1. Activation of Sorbent
2. Removal of Activation Solvent
3. Application of Sample
4. Removal of Interferences (I)
5. Elution of Concentrated, Purified Analytes (A)



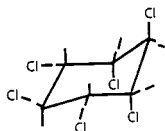
For RP-SPE, methanol is used to condition or wet the sorbent surface, thereby activating the octadecyl moiety, hence forcing it to be receptive to

a van der Waals type of intermolecular interaction between the analyte and the C_{18} moiety. This phenomenon is shown below for the isolation of *n*-butyl phthalate on a C_{18} chemically bonded sorbent (3):



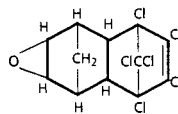
The OCs studied in this exercise are lindane, endrin, and methoxychlor. Lindane (γ -BHC) is synthesized via chlorination of benzene in the presence of ultraviolet light. This forms a mixture of BHC isomers which are identified as α , β , γ , δ , and ϵ . Selective crystallization isolates the γ isomer whose aqueous solubility is 7.3–10.0 ppm and is the most soluble of the BHC isomers. Endrin is a member of the cyclodiene insecticides and is synthesized using Diels–Alder chemistry. Methoxychlor belongs to the *p,p'*-DDT category and structurally differs from DDT in substitution of a methoxy group in place of a chloro group para to the central carbon. Methoxychlor's aqueous solubility is 0.1–0.25 ppm and exceeds that of *p,p'*-DDT by a factor of 100 (4). Molecular structures and correct organic nomenclature of these three representative OCs are shown in the following:

gamma-BHC



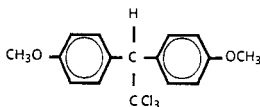
γ -1,2,3,4,5,6-Hexachlorocyclohexane

Endrin



1,2,3,4,10,10-Hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo, endo-5,8-dimethanonaphthalene

p,p'-Methoxychlor



1,1,1-Trichloro-2,2-bis (*p*-methoxyphenyl) ethane

Experimental

Gas chromatograph that incorporates an electron-capture detector

Preparation of Chemical Reagents

NOTE: ALL REAGENTS USED IN THIS ANALYTICAL METHOD CONTAIN HAZARDOUS CHEMICALS! WEAR APPROPRIATE EYE PROTECTION, GLOVES, AND PROTECTIVE ATTIRE. USE OF CONCENTRATED ACIDS AND BASES SHOULD BE DONE IN THE FUME HOOD.

Chemicals/Reagents Needed per Group

- 1 1000 ppm each of lindane, endrin, and methoxychlor stock standard solution dissolved in iso-octane. This is a solvent available in ultrahigh purity, which is an important requirement in trace environmental analysis.
- 1 20 ppm of an internal standard; available candidates include 4-hydroxy-2,4,6-trichlorobiphenyl, 3,4,3',4'-tetrachlorobiphenyl, 1,2-dibromo-3-chloropropane, and β -BHC
- 1 vial containing approximately 30 mL methanol for conditioning the RP-SPE sorbent
- 1 vial containing approximately 30 mL iso-octane

Preliminary Planning

Because there are two sample preparation methods to be implemented, assemble as a group at the beginning of the laboratory session and decide who does what. Once all results are obtained, the group should reassemble and share all analytical data.

Selection of a Suitable Internal Standard

The most appropriate IS needs to be selected from the above list of candidates. Consult with your laboratory instructor and proceed to inject one or more ISs and base your decision on an interpretation of the chromatogram.

Procedure for Calibration and Quantitation of the GC-ECD

1. Prepare the necessary primary and secondary dilution standards. The range of concentration levels for the unknowns are between 100 and 1000 pg/ μ L (ppb). For example, take a 100- μ L aliquot of the 1000-ppm stock and add to a 10-mL volumetric flask previously half-filled with iso-octane. Adjust to the calibration mark and label "10 ppm L,E,M (iso-octane), primary dilution." A 1 : 10 dilution of this primary dilution standard gives a second-

ary dilution which should be labeled “1 ppm L,E,M (iso-octane), secondary dilution.” Use the secondary dilution to prepare a series of working calibration which cover the range of concentrations in the ppb domain as discussed above.

2. Prepare a set of working calibration standards to include an ICV that brackets the anticipated range for the unknowns. To each calibration standard, add 50 μL of 20 ppm IS so that the **concentration of IS in each calibration standard is identical and at 1.0 ppm.**
3. Retrieve the Turbo method titled “LEMIS,” which stands for lindane, endrin, methoxychlor, internal standard mode of calibration; allow sufficient instrument equilibration time. Write a sequence encompassing the calibration standards, ICV, and unknowns. Save the sequence as a file with the name, for example, “G#0317” (group #, March 17th). Begin to inject a 1- μL aliquot of each working standard. Initially inject iso-octane, then inject in the order lowest to highest concentration level. This order is important because it prevents carryover from one standard to the next.
4. Update the calibration for the “LEMIS” method and check with your instructor as to the acceptability of the calibration. If found acceptable, proceed to the analysis once samples have been prepared using both extraction methods. *Be sure to add the same amount of IS to each unknown extract, as was done for the calibration standards.* Because the instrument has been calibrated and updated, the report will include an accurate readout of concentration in a tabular format.

Procedure for Performing μLLE and RP-SPE

5. Place exactly 40 mL of unknown sample into a 42-mL vial and extract using 2 mL iso-octane in a similar manner as you did for the BTEX/THMs exercise. This time, however, add twice the amount of IS that you added for the preparation of the calibration standards so that the concentration of IS remains identical to that for all other standards and samples.
6. Place exactly 40 mL of unknown sample into the 70-mL SPE reservoir which sits atop a previously conditioned C_{18} sorbent according to specific instructions given to you by your laboratory instructor. Add distilled, deionized water (DDI) to the reservoir so as to fill to near capacity. Pass the aqueous sample through the cartridge which contains approximately 200 mg of C_{18} chemically bonded silica gel. Use a wash bottle which contains DDI to rinse the residual sample from both the reservoir and the cartridge.

Place a second SPE cartridge which is filled with anhydrous sodium sulfate beneath the sorbent cartridge. The second SPE cartridge containing anhydrous Na_2SO_4 is used to remove residual moisture from the eluent. Into the manifold place a 1.0-mL volumetric flask as an eluent receiver and elute with two successive 500- μL aliquots of iso-octane. Add the same amount of IS as used for the calibration standards, then adjust to a final volume of 1.0 mL. Transfer to a separate container if necessary.

7. Inject a 1- μL aliquot of the sample extract which also contains the IS into the GC-ECD. At this point, the "LEMIS" method should have had its calibration updated.
8. Continue to make injections into the calibrated GC-ECD until all samples have been completed. You may want to make replicate injections of a given sample extract.

For the Report

Include all calibration plots and calculate the correlation coefficient for the calibration plot. Calculate the precision and accuracy for the ICV, which should have been injected in triplicate. Report on the concentration of each unknown sample. Construct a table which shows the respective concentrations for the unknowns for each of the two methods. Recall that the final extract volume from μLLE was 2 mL and that from SPE was 1 mL. Take this into account when comparing the two methods. Which sample preparation method is preferable? Give reasons for your preference and support this with analytical data.

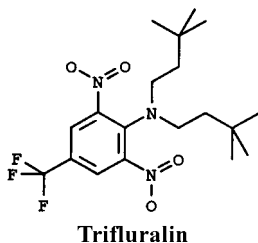
References

1. Perry J. Introduction to Analytical Gas Chromatography. New York: Marcel Dekker, Inc., 1981, pp 164–175. (This is one of the better discussions of the principles behind the operation of the ECD up through technological development of that time.)
2. Methods for Organic Chemical Analysis of Municipal and Industrial Wastewater, EPA-600/4-82-057, July, 1982. Cincinnati, OH: Environmental Monitoring and Support Laboratory, 1982.
3. SPE Sample Preparation. J.T. Baker Chemical Company, 1984, p 8.
4. Lee H, A Chau, F Kawahara. Organochlorine pesticides. In: Chau and Afghan, eds. Analysis of Pesticides in Water. Boca Raton, FL: CRC Press, 1982, Vol. II, Chap. 1. (This work is part of a three-volume series and is a good introduction to pesticide residue analysis even though it is outdated in the use of packed instead of capillary GC columns.)

**DETERMINATION OF THE HERBICIDE RESIDUE TRIFLURALIN IN CHEMICALLY TREATED
LAWN SOIL BY GAS CHROMATOGRAPHY USING SOLID-PHASE EXTRACTION
TECHNIQUES**

Background and Summary of Method

The persistence of trace residue levels of pesticides and herbicides in the environment has been cause for continued concern since the early sixties, when it became apparent that these residues were detrimental to wildlife and possibly to human health. The benefits of using DDT gradually gave way to the increasing risk of continued use and led to the banning of its use. Herbicides, however, do not appear to present such a high risk to the environment and continue to find widespread use. The chlorophenoxy acid herbicides are not directly amenable to GC and must first be chemically converted to their more volatile methyl esters prior to analysis using GC. Trifluralin or, according to IUPAC organic nomenclature, α,α,α -trifluoro-2,6-dinitro-*N,N*-dipropyl-*p*-toluidine, is commonly one of the active pre-emergent herbicide ingredients in some lawn treatment formulations. Consider the molecular structure of trifluralin:



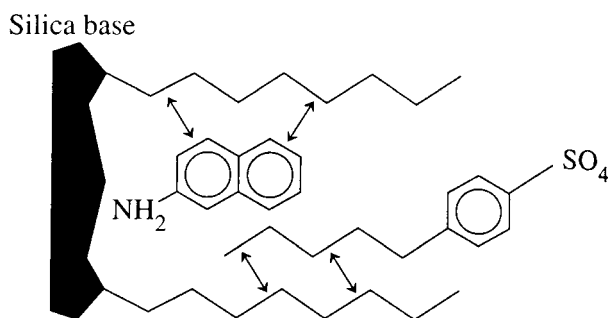
With reference to the molecular structure for trifluralin, the presence of electronegative heteroatoms, such as fluorine combined with two nitro substituents on the benzene ring, would make the organic compound highly sensitive to the electron-capture detector (ECD) provided that the substance is sufficiently vaporizable and therefore amenable to GC. With a boiling point of 139°C, trifluralin is appropriately classified as a semivolatile, neutral organic and could be isolated by conventional sample preparation techniques such as liquid-liquid extraction (LLE) (1, 2).

The assay for the commercially available formulation that was dispersed over the lawn whose soil beneath has been sampled is given as follows:

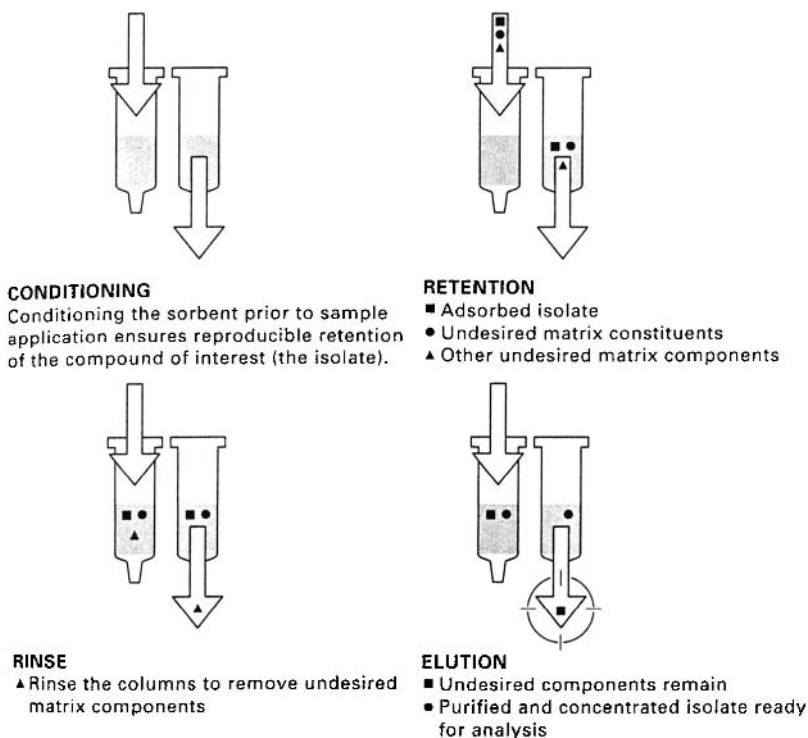
Ingredient	%	Ingredient	%
Total Nitrogen	20	Chlorine (not more than)	3
Trifluralin (<i>N,N</i> -dipropyl)	0.82	Ammonical nitrogen	1.17
Urea nitrogen	18.83	Trifluralin(<i>N</i> -butyl, <i>N</i> -ethyl)	0.43
Sulfur	1.2	Soluble potash	3
Available phosphate	3	Inert	98.75

Solid-Phase Extraction

Solid-phase extraction (SPE) techniques provide an alternative to LLE whereby a chemically bonded silica gel is packed into micro-columns or impregnated into disks and used to isolate and recover semivolatile organic contaminants from various environmental samples (3, 4). A chemically neutral organic compound originally dissolved in water is thermodynamically unstable, and if an aqueous solution containing this compound is allowed to contact a hydrophobic surface, a much stronger van der Waals type of intermolecular interaction causes the molecules of the analyte to “stick” to the surface and thus effectively be removed from the aqueous media. A relatively small volume of a nonpolar or sometimes polar solvent provides enough hydrophobic interaction to then remove (elute in a chromatographic sense) the analyte molecules. The following is a schematic for the interaction of analyte molecules 2-naphthylamine and hexylbenzene sulfonate, with a C₈-bonded silica:



The SPE technique is performed in a stepwise manner as follows:

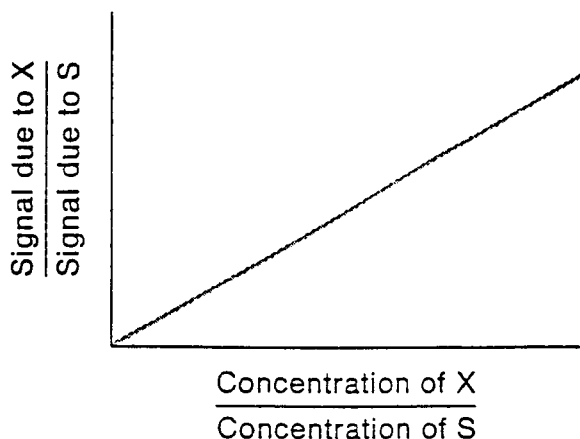


Trifluralin, which might be present in lawn-treated soil, will be initially extracted into methanol. The methanol extract will be diluted with distilled, deionized water (DDI) and the aqueous solution transferred to a 70-mL SPE reservoir on top of a conditioned C_{18} -bonded silica sorbent. The sorbent cartridge will be eluted with high-purity iso-octane. The iso-octane eluent is dried by passing it through a second SPE cartridge directly into a 1.0-mL volumetric receiver. An internal standard is then added and the eluent brought to a final volume of 1.0 mL. A 1–2- μ L aliquot of the eluent can then be directly injected in a C-GC-ECD (Autosystem GC). The concentration of trifluralin in the eluent can be determined following establishment and verification of the instrument calibration.

Internal Standard Mode of Calibration

In addition to external standard and standard addition, the last principal mode of calibration is the called internal standard. This mode of calibration

should be used when there exists variability in sample injection volume, when there is concern about the lack of instrument stability, and when there is unavoidable sample loss. Instrumental response becomes related then to the ratio of the unknown analyte X to that for the internal standard S instead of related only to the unknown analyte. If some X is lost, one can assume that some S would be lost as well. This preserves the ratio $[X]/[S]$. For extraction methods, the internal standard (IS) is added to the final extract just prior to adjusting the final volume. Selecting a suitable IS is not trivial. It should possess similar physical and chemical properties to the analyte of interest and not interfere with the elution of any of the analytes that need to be identified and quantitated. The IS should be within the same concentration range as for the calibration standards and at a fixed concentration. A calibration curve for the IS mode is shown as follows (5):



Experimental

Preparation of Chemical Reagents

NOTE: ALL REAGENTS USED IN THIS ANALYTICAL METHOD CONTAIN HAZARDOUS CHEMICALS! WEAR APPROPRIATE EYE PROTECTION, GLOVES, AND PROTECTIVE ATTIRE. USE OF CONCENTRATED ACIDS AND BASES SHOULD BE DONE IN THE FUME HOOD.

Chemicals/Reagents/Accessories Needed per Group

- 1 10 mL iso-octane, suitable for trace pesticide residue analysis
- 1 100 mL methanol, suitable for trace pesticide residue analysis

- 10 SPE cartridges packed with approximately 200 mg C₁₈-bonded silica
- 10 Empty SPE cartridges loosely packed with approximately 500 mg anhydrous sodium sulfate
- 1 Vac Master SPE Vacuum Manifold (International Sorbent Technology, Ltd.) connected to a vacuum pump via a water trap
- 10 1.0-mL glass volumetric flasks with ground glass stoppers
- 1 10- μ L microliter syringe (Hamilton Co.) for injection into the GC
- 1 10 ppm trifluralin reference stock standard dissolved in iso-octane
- 1 10 ppm 1,2,4-trichlorobenzene (IS) dissolved in MeOH or methyl*tert*-butyl ether (MTBE)

Preparation of the Working Calibration Standards

From the reference stock solution of trifluralin in iso-octane, prepare a series of working calibration standards which cover the range of concentration levels between 10 and 1000 ppb of trifluralin in high purity iso-octane. Each working standard should also contain the IS at a concentration level that should fall within the range of concentrations for the calibration standards. This level should be identical among all standards and sample extracts. Use the table below to guide you in preparing your calibration standards.

Standard No.	# μ L 10 ppm trifluralin	# μ L, 10 ppm IS	V(T) (mL)	Conc. trifluralin (ppb)
0	0	50	1.0	0
1	25	50	1.0	250
2	50	50	1.0	500
3	100	50	1.0	1000
ICV	40	50	1.0	400

Establishing the Calibration

Retrieve the method titled “Triflu.mth,” **create** a sequence file, and **download** the sequence. **Turn off** the nitrogen makeup gas to the ECD and **measure the split ratio**. **Adjust to a ratio of between 15 and 20 to 1**. **Turn the makeup on** after you make the split ratio measurements.

Inject approximately 1 μ L of each working calibration standard and inject the ICV in triplicate using the 10- μ L microliter liquid-handling syringe. **Update** the Turbochrom method titled “Triflu.mth” with the new calibration standards data using the Graphic Editor within Turbochrom. The precision and accuracy data for the ICV can be obtained by retrieving

the Graphic Editor and bringing up the raw file for each ICV and print the tabular formatted report for that particular sample.

Isolating Trifluralin from Lawn-Treated Soil Using SPE Techniques

A Vac Master SPE Vacuum Manifold which should be connected to a vacuum pump via a water trap should be available at the workbench for each of the four workstations. **Condition the C₁₈ sorbent by passing 2 mL of MeOH through it.** Attach a 70-mL polypropylene reservoir to the top of the SPE cartridge and fill with DDI to approximately two-thirds full.

Place 0.25 g of lawn-treated soil into each of three 50-mL beakers, add 10 mL of methanol (pesticide residue grade) to each beaker, and use a glass stirring rod or plastic magnetic stirring bar to stir this mixture for 5 min. Let stand for another 5 min; then decant the supernatant liquid through a Pasteur pipette which contains nonsilanized glass wool to remove large particulates into a clean beaker. Transfer the liquid to the 70-mL reservoir. Turn on the vacuum pump and pass the contents of the reservoir through the C₁₈ sorbent cartridge. After the contents of the reservoir have passed through the sorbent, rinse the reservoir and cartridge with DDI.

Remove the surface moisture with a tissue or equivalent and attach a second SPE cartridge which contains anhydrous sodium sulfate beneath the C₁₈ sorbent cartridge which contains the retained trifluralin. Elute the sorbent with two 500- μ L aliquots of iso-octane into a 1.0-mL volumetric flask. Remove the receiving volumetric flask from the apparatus and adjust to the calibration mark on the flask with iso-octane.

Inject 1 μ L of the dried iso-octane eluent into the C-GC-ECD. Repeat for the other two samples.

For the Report

Include all calibration plots, correlation coefficients, and precision and accuracy estimates of the ICV and report on the concentration of trifluralin in the soil in mg/kg (ppm).

References

1. Budavari S, ed. The Merck Index. 12th ed. Rahway, NJ: Merck and Co., 1996, p 9812.
2. Appropriate conventional methods for determining semivolatile organics include: EPA Method 508 and 608; Standard Methods for the Examination of Water and Wastewater, 19th ed., 1995; Test Methods for Evaluating Solid Waste, SW-846, EPA Office of Solid Waste, Method 8080.

3. Hagen D, et al. *Anal Chim Acta* 236:157–164, 1990.
4. Loconto PR. *LC–GC* 9(7):460–465, 1991; 9(11):752–760, 1991. Varian Sample Preparation Products, Harbor City, CA and Waters Associates Inc., Milford, MA, publish extensive applications bibliographies and periodically update these.
5. Harris D. *Quantitative Chemical Analysis*. 3rd ed. San Francisco: Freeman and Co., 1991, pp 604–605. Also refer to Chapter 2 on the use of the internal standard mode of calibration.

**DETERMINATION OF PRIORITY POLLUTANT VOLATILE ORGANIC COMPOUNDS (VOCs)
IN GASOLINE-CONTAMINATED GROUNDWATER USING STATIC HEADSPACE (HS)
SAMPLE PREPARATION TECHNIQUES COMBINED WITH GAS CHROMATOGRAPHY**

Background and Summary of Method

Benzene, toluene, ethylbenzene, *para*-, *meta*-, and *ortho*-xylenes, collectively referred to as BTEX, constitute some of the most environmentally detrimental organic compounds that have made their way into groundwater primarily due to gasoline spills and underground storage tank leakage. In preparing for this experiment, this author has contaminated groundwater with gasoline and has observed not only the six BTEX components but also a large and early eluting peak that matches the retention time of methyl *tert*-butyl ether (MTBE), a gasoline additive and a known groundwater contaminant. BTEX compounds comprise around 20–30% of gasoline and have an appreciable solubility in water in contrast to aliphatic hydrocarbons. EPA Methods 601 and 602 comprise the real workhorse approaches to trace VOC analyses in wastewaters. These methods use dynamic headspace sampling coupled to GC with electrolytic conductivity (601) and photoionization (602) detection (1). EPA Method 502.2 is a high-resolution capillary column method with both detectors cited above connected in series and the method provides monitoring capabilities for over 60 VOCs that could be found in municipal drinking water supplies (2). An alternative to dynamic headspace (commonly referred to as purge and trap) is static headspace GC techniques (3, 4).

This alternative to purge and trap takes advantage of the volatility exhibited by VOCs whereby the air remaining in a sealed vial above a liquid which is known as static headspace (HS) is sampled with a gas-tight syringe and directly injected into the GC–FID for carbon-containing VOCs. This technique is called manual HS–GC as distinguished from automated HS–GC techniques. We will focus on a subcategory of VOCs, namely BTEX in this experiment.

Of What Value Is This Experiment?

This exercise affords the student an opportunity to further utilize gas chromatography to conduct trace quantitative analysis of gasoline-contaminated groundwater samples. Even though extensive quality control is necessary in laboratories that conduct private and regulated trace analyses, students should be aware of the following. Whenever a sample preparation method is used, there is most always loss of analyte due to sample handling, and if the method involves phase distribution equilibria, **some analyte is lost between phases**. This is particularly important when conducting VOC analyses due to volatility losses of the analytes. It thus becomes imperative that, prior to actual sample analysis, **a recovery study be undertaken to evaluate these losses**. An analyst cannot assume that every method yields a 100% recovery of every organic compound! Recovery studies thus become a major part of a good quality assurance program for the trace environmental analysis laboratory.

The method titled “BTEX.mth” will be retrieved from Turbochrom, External standard calibration curves will be generated using HS-C-GC-FID (headspace capillary gas chromatography with flame ionization detection). An aqueous environmental sample that has been contaminated with gasoline will be available and analyzed for traces of BTEX.

Experimental

Preparation of Chemical Reagents

NOTE: ALL REAGENTS USED IN THIS ANALYTICAL METHOD CONTAIN HAZARDOUS CHEMICALS! WEAR APPROPRIATE EYE PROTECTION, GLOVES, AND PROTECTIVE ATTIRE. USE OF CONCENTRATED ACIDS AND BASES SHOULD BE DONE IN THE FUME HOOD.

Chemicals/Reagents Needed per Group

- 1 Neat benzene
- 1 Neat toluene
- 1 Neat ethylbenzene
- 1 Neat xylenes: ortho, meta, and para
- 1 2000 ppm BTEX, certified reference standard, dissolved in MeOH
- 1 40 mL of a gasoline-contaminated groundwater for BTEX determination
- 1 40 mL of an unknown sample prepared by the staff for BTEX determination

Items/Accessories Needed per Student or per Group

- 1 22-mL glass headspace vials with PTFE/silicone septa and aluminum crimp-top caps
- 1 Crimping tool for headspace vials
- 1 0.5 or 1.0 cm³ capacity gas-tight syringe for headspace sampling and direct injection (Precision Sampling Corp.)
- 1 Heating block assembly that accepts a 22-mL HS vial and allows for measurement of the block temperature

Preliminary Planning

At the onset of the laboratory period, assemble as a group and decide who is going to do what. Assign specific tasks to each member of the group. Once all results are obtained, the group should reassemble and share all analytical data.

Procedure for BTEX Instrumental Analysis Using HS Techniques

1. Using the 2000-ppm BTEX stock reference solution, prepare a series of calibration standards in which the BTEX is present in 10 mL DDI contained in a 22-mL HS vial with PTFE/silicone septa and aluminum crimp caps. Refer to the calibration in Step 2 for reference as you prepare a series of working calibration standards for HS-GC analysis. Following the development of a calibration curve, inject the ICV (only one injection per vial is acceptable in HS-GC) and then one or more of the gasoline-contaminated aqueous samples.
2. Prepare a series of working calibration standards and ICVs according to the following table:

Standard No.	# μ L 2000 ppm BTEX in MeOH	Final volume (mL)	Conc. BTEX (ppm)
Blank	0	10	0
1	20	10	4
2	40	10	8
3	80	10	16
ICV-1	30	10	6
ICV-2	30	10	6
ICV-3	30	10	6

3. Place the indicated aliquot of 2000 ppm BTEX (MeOH) into a 22-mL HS vial containing 10 mL of DDI and seal promptly using the

- crimping tool. Place the vial into the heated block; the temperature should be approximately 85°C.
4. Retrieve the method “BTEX” from Turbo, open a new sequence file, and name the raw data file according to the following example: “G116” (group 1, 16th of the month). Save the sequence file and name it in a manner similar to the following example: “G10316” (group 1, March 16th). Download the method (BTEX.mth) and the sequence file (e.g., G10316.seq).
 5. Make manual injections of approximately 0.25 cm³ of headspace using a gas-tight syringe (refer to the next section). After the three calibration standards have been run, update the calibration method within Turbo. **Ask your lab instructor for help in updating the calibration within the method.** Observe the calibration curve and note the value of the square of the correlation coefficient. Discuss with your instructor whether this calibration is acceptable.
 6. After the instrument has been properly calibrated, proceed to inject the headspace for the three ICVs and any and all gasoline-contaminated samples. Obtain the interpolated values for your three ICVs and for any and all samples. **Obtain assistance from staff in getting a hardcopy of your results.**

Technique for Conducting a Manual Headspace Sampling and Direct Injection Using a Gas-Tight Sampling Microsyringe

If a heater block is available, place the sealed and capped 22-mL headspace vial into the block and allow time for the vial to equilibrate before sampling. A water bath (i.e., a large beaker which is half-filled with water) could serve as a constant-temperature environment for headspace sampling. Insert the gas-tight syringe with the valve in the “on” position by penetrating the septum seal and withdraw a 0.25-cm³ aliquot of headspace. Be careful not to withdraw any liquid. Immediately close the on–off valve to the syringe while positioned within the headspace. Position the syringe into the injection port, open the syringe valve, and transfer the 0.5-cm³ aliquot into the GC.

For the Report

Include the following:

1. A three-point external calibration plot for each chromatographically resolved BTEX analyte with corresponding correlation coefficient; note that Turbochrom finds the square of the correlation coefficient
2. Tabular format which includes results for the ICVs and samples

3. The coefficient of variation for the ICVs
4. The relative error for the ICVs
5. A representative gas chromatogram for the separation

References

1. Methods for Organic Chemical Analysis of Municipal and Industrial Wastewaters, EPA-600/4-82-057, July, 1982. Cincinnati, OH: Environmental Monitoring Systems Laboratory, 1982.
2. Methods for the Determination of Organic Compounds in Drinking Water, EPA/600/4-88/039, December, 1988. Cincinnati, OH: Environmental Monitoring and Support Laboratory, 1988.
3. Kolb B, L Ettre. Static Headspace Gas Chromatography: Theory and Practice. New York: Wiley-VCH, 1997. This book is one of the most complete treatments of static headspace GC available.
4. McNally M, R Grob. Am. Lab. 20–33, January 1985; a somewhat comprehensive review with 143 references.

DETERMINATION OF PRIORITY POLLUTANT VOLATILE ORGANIC COMPOUNDS (VOCs) in WASTEWATER USING MICRO-LIQUID-LIQUID EXTRACTION (μ LLE) AND STATIC HEADSPACE (HS) SAMPLE PREPARATION TECHNIQUES COMBINED WITH GAS CHROMATOGRAPHY

Background and Summary of Method

Benzene, toluene, ethylbenzene, *para*-, *meta*-, and *ortho*-xylenes, collectively referred to as BTEX, constitute some of the most environmentally detrimental organic compounds that have made their way into groundwater primarily due to gasoline spills and underground storage tank leakage. Another groundwater contaminant in recent years has been methyl *tert*-butyl ether (MTBE). BTEX compounds comprise around 20–30% of gasoline and have an appreciable solubility in water in contrast to aliphatic hydrocarbons. EPA Methods 601 and 602 comprise the real workhorse approaches to trace VOCs analyses in wastewaters. These methods use dynamic headspace sampling coupled to GC with electrolytic conductivity (601) and photoionization (602) detection (1). Trihalomethanes (THMs), which consist of chloroform, dichlorobromomethane, dibromochloromethane, and bromoform, have been found in drinking water which has been disinfected using chlorine. EPA Method 502.2 is a high-resolution capillary column method with both detectors cited above connected in series and provides monitoring capabilities for over 60 VOCs that could be found in municipal drinking water supplies (2). An alternative to dynamic headspace (commonly referred to as purge and trap) is static headspace GC techniques (3).

We will take a more simplified approach to trace VOC analysis which utilizes our limited sample preparation and instrumentation capabilities. If a suitable extraction solvent can be found (i.e., one which does not interfere with the VOCs to be identified and quantitated by gas chromatography), then the analytes of interest can be isolated and concentrated from the environmental sample matrix via the μ LLE technique (4, 5). A 40-mL sample which might contain extract is injected into a GC-FID to determine BTEX. The extraction is performed on a sample that might contain THMs, such as chlorine-disinfected water and 2 μ L of extract is injected into a GC-ECD to determine the THMs. This method is to be used for quantitative analysis of gasoline-contaminated groundwater samples. Typical levels of contamination are in the low parts per million (ppm) concentration range. A severe limitation to the μ LLE method is the possible formation of emulsions when applied to wastewaters which could have an appreciable surfactant concentration level.

An alternative to liquid-liquid extraction that is available to use takes advantage of the volatility exhibited by VOCs whereby the air remaining in a sealed vial above a liquid which is known as static headspace (HS) is sampled with a gas-tight syringe and directly injected into the GC-FID for carbon-containing VOCs and into the GC-ECD for chlorinated VOCs such as THMs. This technique is called manual HS-GC, as distinguished from automated HS-GC techniques.

Of What Value Is This Experiment?

This exercise affords the student an opportunity to further utilize gas chromatography in combination with two different sample preparation methods. Whenever a sample preparation method is used, there is most always loss of analyte due to sample handling, and if the method involves phase distribution equilibria, some analyte is lost between phases. This is particularly important when conducting VOCs analyses due to volatility losses of the analytes. It thus becomes imperative that, prior to actual sample analysis, a recovery study be undertaken to evaluate these losses. An analyst cannot assume that every method yields a 100% recovery of every organic compound! Recovery studies thus become a major part of a good quality assurance program for the trace environmental analysis laboratory.

Previously created methods will be retrieved from Turbochrom. External standard calibration curves will be generated using both sample preparation techniques. An aqueous environmental sample that has been contaminated with gasoline will be analyzed for traces of BTEX using both μ LLE and HS techniques.

Comparison of two dependent averages is a statistical procedure which helps to determine whether two different analytical methods give the same average result for a given sample. If one analyzes each of a series of samples by the two methods, a pair of results for each sample will be obtained. The difference between these two results for each pair will reflect only the difference in the methods; the effects of concentration will be eliminated. The following equation is used for the t test on paired data (6):

$$t = \frac{\bar{d}}{s_d} \sqrt{n}, \quad s_d = \frac{\sum d^2 - (\sum d)^2/n}{n - 1}$$

where d is the difference in each pair of values, $\bar{d}$ is the average difference in the pairs of values (use the absolute value), n is the number of pairs of values, df is the degrees of freedom associated with t , and s_d is the standard deviation of the differences between the pairs of observations.

A comparison of the calculated t with that from the table of the student's t is then made. If $t(\text{calculated}) > t(\text{from table at the desired level of significance})$, then both methods do not give the same result. If $t(\text{calculated}) < t(\text{table})$, then it is statistically valid to assume that both methods are equivalent.

Experimental

Preparation of Chemical Reagents

NOTE: ALL REAGENTS USED IN THIS ANALYTICAL METHOD CONTAIN HAZARDOUS CHEMICALS! WEAR APPROPRIATE EYE PROTECTION, GLOVES, AND PROTECTIVE ATTIRE. USE OF CONCENTRATED ACIDS AND BASES SHOULD BE DONE IN THE FUME HOOD.

Chemicals/Reagents Needed per Group

- 1 Neat benzene
- 1 Neat toluene
- 1 Neat ethylbenzene
- 1 Neat xylenes
- 1 Neat hexane
- 1 Neat hexadecane
- 1 Neat dichloromethane (methylene chloride)
- 1 Approximately 5000 ppm stock BTEX standard (refer to actual label for exact values)

- 1 40 mL of a gasoline-contaminated groundwater for BTEX determination
- 1 40 mL of an unknown sample prepared by the staff for BTEX determination

Items/Accessories Needed per Student or per Group

- 1 42-mL glass vials with crew caps and PTFE/silicone septa
- 1 22-mL glass headspace vials with PTFE/silicone septa and aluminum crimp-top caps
- 1 Crimping tool for headspace vials
- 1 Microsyringe with Chaney adapter (Hamilton Co.) for injection of liquid extracts 0.5 or 1.0 cm³ capacity gas-tight syringe for headspace sampling and direct injection (Precision Sampling Corp.)

Preliminary Planning

At the onset of the laboratory period, assemble as a group and decide who is going to do what. Assign specific tasks to each member of the group. Once all results are obtained, the group should reassemble and share all analytical data.

Procedure for BTEX Instrumental Analysis Using μ LLE Techniques

Selecting the Most Suitable Extraction Solvent

1. Place one small drop of each of the neat BTEX liquids into approximately 10 mL of hexane. Inject 1 μ L into the GC-FID and interpret the resulting chromatogram. Repeat for dichloromethane and then for hexadecane. From these observations, select the most appropriate extraction solvent, then proceed to prepare calibration standards.

Preparation of the Primary Dilution Standard and Working Calibration Standards

2. Using a clean and dry glass pipette (volumetric), transfer 1.0 mL of the 5000-ppm BTEX to a 10-mL volumetric flask which has been previously half-filled with the most suitable solvent that you chose earlier. Adjust to the calibration mark with this solvent and label as, for example, "500 ppm BTEX." This is what EPA methods call a "primary dilution standard" because it is the first dilution that the analyst prepares from a given source which has a higher concentration.

3. Prepare a series of working calibration standards according to the following table:

Standard No.	# mL 500 ppm BTEX	Final volume (mL)	Conc. BTEX (ppm)
1	1	10	50
2	2	10	100
3	4	10	200
4	8	10	400
5	—	—	500
ICV	5	10	250

4. Retrieve the method BTEX from Turbo, open a new sequence file, and name the raw data file according to the following example: “G116” (group 1, 16th of the month). Save the sequence file and name it in a manner similar to the following example: “G10316” (group 1, March 16th).
5. Inject 1- μ L aliquots of all calibration standards and inject the ICV in triplicate. Update the calibration method within Turbo. **Ask your lab instructor for help in updating the calibration within the method.** Observe the calibration curve and note the value of the square of the correlation coefficient. Discuss with your instructor whether this calibration is acceptable or not.
6. After the instrument has been properly calibrated, proceed to inject the extracts from the μ LLE method for the unknown contaminated samples (refer to the procedure below). Obtain the interpolated values from the external standard mode of calibration.

Procedure for BTEX Instrumental Analysis Using HS Techniques

Using the 500-ppm BTEX stock reference solution, prepare a series of calibration standards in which the BTEX is present in 10 mL DDI which is contained in a 22-mL HS vial with PTFE/silicone septa and aluminum crimp caps. Refer to the above calibration for reference as you prepare a series of working calibration standards for HS–GC analysis. Following the development of a calibration curve, inject the ICV (only one injection per vial is acceptable in HS–GC), then inject the headspace above the aqueous samples. Following the development of a calibration curve, inject the ICV (only one injection per HS vial is valid) and the contaminated aqueous samples.

Procedure to Conduct a μ LLE

Once the most appropriate extraction solvent has been selected, the aqueous sample which contains dissolved BTEX or THMs can be extracted. To a clean 42-mL glass vial with a PTFE/silicone septum and screw cap, add 40 mL of aqueous sample. Pipette 2.0 mL of extraction solvent; place the septum and cap in place. Shake for 1 min and let stand for at least 5 min until both phases clearly separate. Using a glass transfer pipette, remove approximately 75% of the extract and place in a small test tube or vial. Inject 1 μ L of extract into the GC-FID or GC-ECD. *Discard the contents of the 42-mL glass vial into the waste receptacles which are located in the laboratory.*

Procedure to Conduct a Headspace Sampling and Direct Injection

If a heater block is available, place the sealed and capped 22-mL headspace vial into the block and allow time for the vial to equilibrate before sampling. A water bath (i.e., a large beaker which is half-filled with water) could serve as a constant-temperature environment for headspace sampling. Insert the gas-tight syringe with the valve in the “on” position by penetrating the septum seal and withdraw a 0.5-cm³ aliquot of headspace. Be careful not to withdraw any liquid. Immediately close the on-off valve to the syringe while positioned within the headspace. Position the syringe into the injection port, open the syringe valve, and transfer the 0.5-cm³ aliquot into the GC.

For the Report

Include all calibration plots; calculate the correlation coefficient for both calibration plots. Determine the precision and accuracy for triplicate injections of the ICV for both calibrations. Report on the concentration in ppm for all unknown samples analyzed. This is an example in which two different methods are used to conduct analysis of the same unknown sample. Use the statistical evaluation for comparing two dependent averages (paired data) to determine whether the two different methods give the same result. You may want to include a representative chromatogram obtained from the gasoline-contaminated sample. **Ask your lab instructor to assist you in obtaining a hardcopy from the laboratory printers.**

References

1. Methods for Organic Chemical Analysis of Municipal and Industrial Wastewaters, EPA-600/4-82-057, July, 1982. Cincinnati, OH: Environmental Monitoring Systems Laboratory.

2. Methods for the Determination of Organic Compounds in Drinking Water, EPA/600/4-88/039, December, 1988. Cincinnati, OH: Environmental Monitoring and Support Laboratory.
3. Kolb B, L Ettre. Static Headspace-Gas Chromatography: Theory and Practice. New York: Wiley-VCH, 1997.
4. Mieure J. J Am Water Works Assoc 69(1):60, 1997.
5. Richard J, G Junk. J Am Water Works Assoc 69(1):62, 1977.
6. Anderson R. Practical Statistics for Analytical Chemists. New York: Van Nostrand, Reinhold, 1987, pp 78-83.

AN INTRODUCTION TO GAS CHROMATOGRAPHY: EVALUATING EXPERIMENTAL PARAMETERS THAT INFLUENCE GAS CHROMATOGRAPHIC PERFORMANCE

Background and Summary of Method

Gas chromatography (GC) is the most widely used instrumental technique for the determination of trace concentrations of organic pollutants found in environmental samples today. Its origins stem from the pioneering work of Martin and Synge in 1941 to the development of open tubular gas chromatographic columns advanced by Golay to the fabrication by Dandeneau at Hewlett-Packard of the flexible fused-silica capillary column (1). It must be recognized however that approximately 20% of all of the organic compounds that exist and could possibly make their way into the environment are amenable to GC techniques without prior chemical modification of the sample (2). Despite this limitation, over 60 organic compounds classified as "volatile organic compounds" (VOCs) have been found in drinking water, groundwater, surface water, and wastewater and are routinely monitored. Over 100 semivolatile or nonvolatile organic compounds have also been found which include phenols, polycyclic aromatic hydrocarbons, mono-, di-, and tri-chloro aromatics and aliphatics, nitro aromatics, polychlorinated biphenyls, organochlorine pesticides, organophosphorus pesticides, triazine herbicides, and phthalate esters, among others.

The theoretical principles that underlie GC is presented in Chapter 4 and in numerous texts and monographs (see Ref. 3 for several examples). Specific methods that incorporate GC as the determinative instrumental technique are to be found in a plethora of analytical methods published by the Environmental Protection Agency (EPA), the American Public Health Association/American Water Works Association/Water Pollution Control Federation (*Standard Methods for the Examination of Water and Wastewater*), and the American Society for Testing Materials (*ASTM, Part 31 Water*).

This exercise introduces the student to those experimental GC parameters that exert a major influence on GC performance. These include (a)

detector selectivity, (b) injection volume versus chromatographic peak shape, (c) the effect of changing the carrier gas flow rate on column efficiency, and (d) the effect of column temperature on chromatographic resolution and analysis time. This exercise affords the student the opportunity to vary these parameters and assess the outcomes. **This is a qualitative analysis exercise only and involves making and recording observations, doing some calculations from information found in the chromatograms.**

Brief Description of Gas Chromatographs in the Hazardous Waste Analysis Lab at Michigan State University

The Perkin-Elmer Autosystem GC consists of a dual-injector port, dual-capillary-column configuration, and dual detectors [flame ionization (FID), electron-capture (ECD)] connected via an analog-to-digital (A/D) interface to a central personal computer “workstation” which is driven by the Turbochrom (PE-Nelson) chromatography data processing software. You will encounter two types of A/D interfaces in the laboratory. The 600 LINK interface provides for both data acquisition and instrument control. The 900 interface provides for data acquisition only.

The *front injector* consists of a split/splitless capillary column type and is connected to a 0.25-mm (i.d.) $\times$ 30-m (length) capillary column (referred to as a narrow-bore-type column). The column is coated with a DB-5 liquid phase (5% phenyl dimethyl siloxane) that is chemically bonded to the inner tubing wall. This type of liquid phase is appropriate for the separation of semivolatile or nonvolatile organic compounds whose boiling points are much greater than 100°C. The optimum volumetric flow rate (i.e., that flow rate which gives a minimum in the *van Deemter* curve) is between 1 and 3 cm³/min. To obtain such a low flow rate, a split vent is required to remove most of the gas. Refer to the instruction manual for setting the split ratio. Typical split ratios are 1 : 25, 1 : 50, or 1 : 100 and this ratio refers to the ratio of gas flow through the column to that through the vent. The outlet end of this column is connected to the inlet to the ECD. This detector requires an additional source of inert gas commonly called “makeup.” The flow rate for the makeup should be approximately 30 cm³/min. Using the Digital Flow Check (refer to the instruction manual), measure the initial flow rate then adjust to the optimum for operation of a “narrow-bore” capillary column.

The *rear injector* consists of a packed column adapted for connection to a 0.53-mm (i.d.) $\times$ 30-m (length) capillary column (referred to as a megabore column). The column is coated with a cyano propyl dimethyl polysiloxane liquid phase that is chemically bonded to the inner tubing wall. This type of liquid phase is appropriate for the separation of volatile organic

compounds (VOCs). The optimum volumetric flow rate is between 5 and 15 cm³/min. The column outlet is connected to the inlet to the FID. This detector does not require makeup gas. The FID, however, requires a 10 : 1 ratio of airflow to hydrogen flow. Conventional flow rates are 300 cm³/min for air and 30 cm³/min for H₂. Once the air/fuel ratio has been established, the FID can be ignited. Sometimes, a slightly fuel rich ratio is necessary to ignite the FID.

Principle of Separation in GC

When two compounds migrate at the same rate through a chromatographic column, no separation is possible. Two compounds that differ in retention times, $t(R)$, or capacity factor, k' , and appear to separate, do so because of differences in their equilibrium distribution constants, denoted by K_D . If K_D is independent of sample size, Gaussian elution bands (i.e., symmetrical peaks) are observed. This is the case of linear elution chromatography. In other words, a plot of the concentration of analyte in the stationary phase to the concentration in the mobile phase yields a straight line whose slope equals K_D . If the amount of analyte increases either by injecting equal volumes of solutions whose concentrations are increasing or by injecting increasing volumes of a solution whose concentration is fixed, nonsymmetrical chromatographic peaks result. K_D is now dependent on the amount of solute, and either peak tailing or peak fronting results. This is the case of nonlinear elution chromatography. Gaussian or symmetrical peak shape is a chief objective when GC is used to perform trace quantitative analysis. The following equations relate the parameters discussed above:

$$k' = \frac{t_R - t_0}{t_0}$$

$$K_D = \beta k'$$

where β is the volume(mobile phase)/volume(stationary phase), t_R is the retention time for a retained peak and t_0 is the retention time for an unretained peak.

Experimental

Gas chromatograph interfaced to a PC that is loaded with a chromatographic software. In our lab, an Autosystem (Perkin-Elmer) is interfaced to a PC workstation that utilizes Turbochrom (PE-Nelson) for data acquisition, processing, and readout.

Preparation of Chemical Reagents

NOTE: ALL REAGENTS USED IN THIS ANALYTICAL METHOD CONTAIN HAZARDOUS CHEMICALS! WEAR APPROPRIATE EYE PROTECTION, GLOVES, AND PROTECTIVE ATTIRE. USE OF CONCENTRATED ACIDS AND BASES SHOULD BE DONE IN THE FUME HOOD.

Accessories to Be Used with the GC per Group

- 1 Digital flow check meter
- 1 GC syringe with a beveled end which includes a Chaney adapter (do not confuse with the blunt-end syringe used for HPLC)
- 1 GC test mix for each of the studies discussed below; refer to the summary at the end of this handout

Procedure

Refer to Table 5.1 for a definition of each of the Turbochrom methods created in support of this experiment. These previously created methods are illustrative of how a chromatography-based software can be used to teach fundamental principles of GC.

Measurement and Adjustment of Carrier Gas Flow Rate and Split Ratio

As you approach the gas chromatograph, you will find it in an operational mode with carrier gas flowing through both capillary columns. If not already set up, retrieve the turbo file titled “FLOWRATE” and download this method. Your first task will be to measure the flow rate of the carrier gas through both capillary columns with the makeup gas off. After turning the makeup gas on, measure the split ratio through the capillary injector using the digital flow check meter. **Record flow-rate data in your lab notebook.**

Once the optimum carrier flow rates have been established, the dual-detector method titled “DETSSENS” can be retrieved from the Turbochrom software, then transferred to the instrument via the interface (a process known as download) and the comparison of detector sensitivity can be undertaken. **Ignite the FID (refer to the Operator’s Manual for the Autosystem GC from Perkin-Elmer for the specific procedure).**

Comparison of the FID Versus the ECD Sensitivity

Allow time for the GC to equilibrate at the column temperature set in the method. Using the manual injection syringe (Hamilton), inject equal micro-liter aliquots of acetone into both injectors. Observe the appearance of a

TABLE 5.1 Summary of Turbochrom Methods to Be Used in This Experiment

Order	Turbo method	Remarks
1	FLOWRATE	Near-ambient column temperature Measure flow rate, split ratio
2	DETSENS	Neat acetone—FID Neat methylene chloride—ECD
3	INJECD	Inject increasing amounts of 10 ppm 1,2,4-trichlorobenzene
	INJFID	Inject increasing amounts of hexadecane at 225°C
4	PLATES	Temperature program: 265 (0.1) to 285 (10.0) at 6 deg/min; multicomponent OC pest mixture
5	TMAX	Isothermal at 285°C
	TMIN	Isothermal at 200°C

retained chromatographic peak found in both chromatograms. You cannot assume that t_R will be identical on both columns. Compare the peak heights from both chromatograms. Inject equal microliter aliquots into both injectors, as earlier, of the specific chlorinated hydrocarbon that is available. **Record your observations** and compare the peak heights as done previously. Each member of the group should have an opportunity to make these sample injections so as to gain some experience with manual syringe injection of organic solvents.

Injection Volume Versus GC Peak Shape

Retrieve the turbo file titled “INJECD” and download. Inject a series of increasing microliter aliquots of a reference solution labeled “10 ppm 1,2,4-trichlorobenzene” into the front capillary injection port. **Observe and record the changes in chromatographic peak shape as the amount of analyte is increased.** Retrieve the Turbo file titled “INJFID” and download. Repeat the series of injections as before using the reference solution labeled “hexadecane” and make these injections into the rear injector. **Observe and record the changes in chromatographic peak shape as the amount of analyte is increased.**

Flow Rate Versus Capillary Column Efficiency

A column's efficiency is determined in a quantitative manner from the chromatogram by measuring the number of theoretical plates, N . The effect of

carrier flow rate on capillary column efficiency is significant in GC and will be examined under isothermal conditions (i.e., at a fixed and unchanging column temperature). Retrieve the turbo file “PLATES” and program this method for a high-flow rate by increasing the head pressure. Save this change in the method and download the method. Turn off the makeup gas and adjust the actual pressure so as to nearly match that which is set in the method and **measure** the flow rate. Turn the makeup back on. Inject 1 μL of the test mix and **observe** the chromatogram. Retrieve the method a second time, reprogram the head pressure to a much lower value. Turn off the makeup, decrease the carrier head pressure, **measure** the new flow rate, turn the makeup gas back on, and then make a second injection using the same volume.

For the carrier gas flow rate which exhibited the highest efficiency, **calculate** the number of theoretical plates using equations from your text. In addition, for the optimum carrier flow rate, choose any pair of peaks and calculate the resolution for that pair.

Column Temperature Versus Capacity Factor

Retrieve the turbo file titled “TMIN” and download the method. Inject approximately 1 μL of the CIHCs test mix at this column temperature of 200°C. **Observe the degree of separation among organochlorine analytes and record your qualitative comments.**

Retrieve the turbo file titled “TMAX” and download the method. Inject the same volume of CIHCs test mix and **observe** the chromatogram when the column temperature has been increased to 285°C.

For the Lab Notebook

Review relevant sections of Chapter 4. Use these concepts to write a brief discussion on how your experimental observations connect to the theoretical relationships discussed in the text.

Address the following:

1. Explain why different GC detectors have different instrument detection limits.
2. If you operated a GC at significantly reduced carrier gas flow rates, predict what you would observe in a gas chromatogram for the injection of organic compounds. What would be the principal cause for these observations?
3. Explain why a symmetrical peak shape is important in gas chromatography.
4. What happens to K_D for a given organic compound when column temperature is varied?

5. How efficient is your GC column? That is, what is the number of theoretical plates? How many plates per meter do you have?
6. How is the phase ratio, β , determined for capillary GC columns?

References

1. Hinshaw J, L Ettre. Introduction to Open Tubular Column Gas Chromatography. ADVANSTAR, 1994. Chapter 1 includes a brief historical account of the significant developments.
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3. Skoog D, J Leary. Principles of Instrumental Analysis. 4th ed. Philadelphia, WB Saunders, 1992, pp 605–625; Harris D. Quantitative Chemical Analysis. 3rd ed. San Francisco: Freeman, 1991, pp 645–662. McNair H, J Miller. Basic Gas Chromatography. New York: John Wiley & Sons, 1997.

A COMPARISON OF SOIL TYPES VIA A QUANTITATIVE DETERMINATION OF THE CHROMIUM CONTENT USING VISIBLE SPECTROPHOTOMETRY AND FLAME ATOMIC ABSORPTION SPECTROSCOPY

Background and Summary of Method

Chromium exists in three oxidation states of which Cr(III) and Cr(VI) are the most stable. Hexavalent Cr is classified as a known human carcinogen via inhalation and Cr(III) is an essential dietary element for humans and other animals. Certain soils which exhibit a strong chemically reducing potential have been shown to convert Cr(VI) to Cr(III). It is possible for the analysis of soils from a hazardous waste site to reveal little to no Cr(VI) via the colorimetric method because this method is selective for Cr(VI) only (1, 2). Because atomic absorption spectrophotometric methods yield total Cr, the difference between analytical results from both methods should be indicative of the Cr(III) content of a given soil type. Both methods will be implemented in this lab exercise and applied to one or more soil types.

This exercise affords students the opportunity to use several instrumental techniques to which they have previously been introduced in the laboratory in order to conduct a comparison of soil types with respect to determining the ratio of Cr(III) to Cr(VI). The exercise includes pH measurement, calibration of an UV–vis spectrophotometer, calibration of an atomic absorption spectrophotometer in the flame mode (FAA), and sample preparation techniques.

Cr(VI) in its dichromate form, $\text{Cr}_2\text{O}_7^{2-}$, reacts selectively with diphenylcarbazide, $\text{C}_6\text{H}_5\text{NHNHCONHNHC}_6\text{H}_5$, in acidic media to form a red–violet color of unknown composition. This selectivity for Cr occurs in the absence of interferences such as molybdenum, vanadium, and mercury. The

colored complex has a very high molar absorptivity at 540 nm. This gives the method a very low detection limit (MDL) for Cr(VI) using an UV-vis spectrophotometer. Flame atomic absorption spectroscopy is also a very sensitive technique for determining total Cr with instrument detection limits (IDLs) as low as 3 ppb (3).

A recent modification to EPA Method 7196 has been published and will be implemented in this lab exercise (4). The method uses a hot alkaline solution (pH 12) to solubilize chromates that are to be found in soils obtained from hazardous waste sites. One portion of the aqueous sample would then be aspirated into the FLAA for a determination of total Cr, whereas diphenylcarbazide dissolved in acetone will be added to another portion, and the absorbance of the red-violet complex will be measured at 540 nm using a visible spectrophotometer. In this manner, both total Cr and Cr(VI) can be determined on the same sample. Thus, the ratio of the concentration of Cr(III) to the concentration of Cr(VI) in a soil sample can be calculated from the data generated in this experiment.

Experimental

Chemical Reagents Needed per Student or Group

NOTE: ALL REAGENTS USED IN THIS ANALYTICAL METHOD CONTAIN HAZARDOUS CHEMICALS! WEAR APPROPRIATE EYE PROTECTION, GLOVES, AND PROTECTIVE ATTIRE. USE OF CONCENTRATED ACIDS AND BASES SHOULD BE DONE IN THE FUME HOOD.

Potassium dichromate stock solution: Dissolve 141.4 mg of dried $\text{K}_2\text{Cr}_2\text{O}_7$ in distilled, deionized water (DDI) and dilute to 1 L (1 mL = 50 μg Cr).

Potassium dichromate standard solution: Dilute 10.00 mL of stock solution to 100 mL (1 mL = 5 μg Cr).

Sulfuric acid, 10% (v/v): Dilute 10 mL of concentrated H_2SO_4 to 100 mL with DDI. Also, 1.8M H_2SO_4 is needed. An aliquot of 10% H_2SO_4 could be used to prepare this solution.

Diphenylcarbazide (DPC) solution: Dissolve 250 mg of 1,5-diphenylcarbazide in 50 mL acetone. Store in an amber bottle. Discard when the solution becomes discolored.

Acetone, CH_3COCH_3 : Use the highest purity available.

Alkaline digestion reagent, 0.28M Na_2CO_3 /0.5M NaOH: Use your knowledge of chemical stoichiometry to calculate the amount of each base needed to prepare a solution of the molarity desired.

Concentrated nitric acid, HNO_3 .

Procedure for Alkaline Digestion

1. Place 2.5 g of a given soil type into a 250-mL beaker. Add 50 mL of the alkaline digestion reagent. Stir at room temperature for at least 5 min and then heat on a hot plate to maintain the suspensions at 90–95°C with constant stirring for about 1 h. Repeat for all other soil types to be studied whose Cr content is to be determined in this experiment.

Note: Heating on a hot plate can cause bumping and lead to splatter and thus loss of analyte.

2. Cool the digestates to room temperature, then filter through 0.45- μ m cellulosic or polycarbonate membrane filters.
3. Adjust the pH to 7.5 using concentrated HNO_3 and dilute with DDI to a final volume of 100 mL. You now have 100 mL of digestate.

Procedure for Conducting Visible Spectrophotometric Analysis

1. Prepare six working standards from careful dilutions of the 5- μ g/mL Cr standard. The range of concentrations should be from 0 to 2 μ g/mL Cr. You should prepare 100 mL of each standard.
2. Prepare an initial calibration verification (ICV) standard which should have its concentration approximately near the mid-range of the calibration. You should prepare 100 mL of the ICV.
3. Prepare a matrix spike and a matrix spike duplicate. The amount of spike should double the concentration found in the original sample. The spike recovery must be between 85% and 115% in order to verify the method.
4. To 45 mL of DDI (this is the method blank), standard, ICV, and digestate, add 1 mL of DPC solution, followed by the addition of 1.8M H_2SO_4 until the pH reaches approximately 2. This should be done in a 125-mL beaker with stirring and immersion of the glass electrode until the desired pH is attained. After cessation of effervescence, dilute the mixture with DDI to 50 mL. Allow the solution to stand from 5 to 10 min. If the solution appears turbid after the addition of DPC, filter through a 0.45- μ m membrane. Store the remaining samples and standards in properly labeled bottles. Use if it is necessary to repeat this analysis.
5. Set the spectrophotometer at 540 nm; correctly set the zero and 100% transmittance settings. Transfer an aliquot of the 50-mL sample to a cuvette. Measure the absorbance of all blanks, stan-

dards and samples. Construct a table in your notebook to facilitate the entry of data.

Procedure for Atomic Absorption Spectrophotometric Analysis or ICP–AES

Refer to SW-846 Methods 7000A (Atomic Absorption Methods) and 7190 (Chromium, atomic absorption, direct aspiration) or Method 6010B (Inductively Coupled Plasma–Atomic Emission Spectrometry) (ICP–AES) for the quantitative determination of chromium at total Cr. In our lab, proceed to prepare calibration standards and ICVs and aspirate these into the flame using the Model 3110 (Perkin-Elmer) atomic absorption spectrophotometer or the Model 2000 (Perkin-Elmer) Plasma ICP–AES. Use the remaining digestates from Step 3 and determine total Cr. the FIAA may need to be set up from its present configuration. Refer to previous exercises and training manuals for the necessary information.

For the Report

Include all calibration data, ICVs, and sample unknowns for both instrumental methods. Perform a statistical evaluation in a manner that is similar to previous experiments. Use EXCEL or “LSQUARES” (refer to Appendix C) or other computer programs to conduct a least squares regression analysis of the calibration data. Calculate the accuracy (expressed as a percent relative error for the ICV) and the precision (relative standard deviation for the ICV) from both instrumental methods. Calculate the percent recovery for the matrix spike and matrix spike duplicate. Report on the concentration of Cr in the unknown soil samples. **Be aware of all dilution factors and concentrations as you perform calculations!**

Find the ratio of the concentration of Cr(III) to that of Cr(VI) in each of the soil samples analyzed and present this value at the end of your report.

References

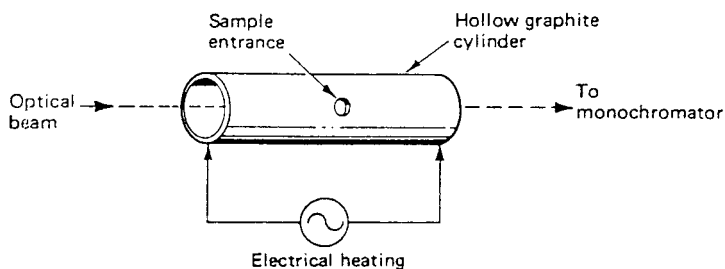
1. Standard Methods for the Examination of Water and Wastewater, 16th ed, APHA, 1985, pp 201–204.
2. Method 7196, Chromium, hexavalent (colorimetric). In: Test Methods for Evaluating Solid Waste Physical/Chemical Methods, SW 846. Washington, DC: Environmental Protection Agency, 1987, Revision 1.
3. Skoog D, J Leary. Principles of Instrumental Analysis. 4th ed. Philadelphia: WB Saunders, 1992, p 223.
4. Vitale R, et al. Am Environ Lab 7(3):1, 8–10, 1995.

**DETERMINATION OF LEAD IN DRINKING WATER USING GRAPHITE FURNACE ATOMIC
ABSORPTION SPECTROSCOPY (GFAA): EXTERNAL STANDARD VERSUS INTERNAL
STANDARD CALIBRATION MODE**

Background and Summary of Method

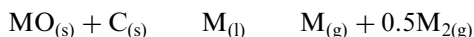
Unfortunately, among the so-called heavy metals which have made their way into the environment, lead (Pb) is considered highly toxic and its presence must be identified and its concentration measured in air, water, and soil. Two principal historical uses which contained Pb were in coatings (e.g., house paint) and in gasoline (e.g., as alkyl lead compounds added to boost octane ratings). The extreme toxicity of Pb has required that instrumental analytical techniques which offer the lowest possible detection limits be used. Pb joins elements such as Hg, Cd, As, and Tl as requiring determinative techniques with the lowest instrument detection limits (IDLs) possible. It is important to recognize the difference between method detection limits (MDLs) and IDLs possible. The MDL incorporates the IDL and is equal to the IDL if and only if there is no sample preparation involved. Sample preparation is more common in trace organics analysis in contrast to trace metals analysis. For example, the IDL for Pb using flame AA is 0.1 ppm, whereas the IDL for Pb using the graphite furnace atomic absorption spectroscopic (GFAA) methods is 1 ppb (1).

When using the furnace technique, a representative aliquot of a sample is placed in the graphite tube in the furnace, evaporated to dryness, charred, and atomized. A greater percentage of available analyte atoms is vaporized and dissociated for absorption in the tube in contrast to the flame. It becomes possible to use smaller sample volumes. Radiation from a given excited element is passed through the vapor containing ground-state atoms of that element. The intensity of the transmitted radiation decreases in proportion to the amount of the ground-state element in the vapor. The metal atoms to be measured are placed in the beam of radiation, which is nearly monochromatic (from a hollow-cathode tube) by increasing the temperature of the furnace. A monochromator isolates the wavelength of the transmitted radiation and a photosensitive device measures the attenuated intensity. Beer's Law of Spectrophotometry applies, as was the case for UV-vis absorption spectrophotometry, and the concentration of the specific element is determined by various modes of analyte calibration in a similar manner to that for UV-vis absorption spectrophotometry. A schematic of an electrothermal atomizer follows:



The tube is usually coated with pyrolytic graphite, which is made by heating the tube in a methane atmosphere. Pyrolytic graphite exhibits a low gas permeability and good resistance to chemical attack and this lengthens the lifetime (i.e., the number of successful firings) of the tube. There is, however, a finite lifetime for each tube in GFAA.

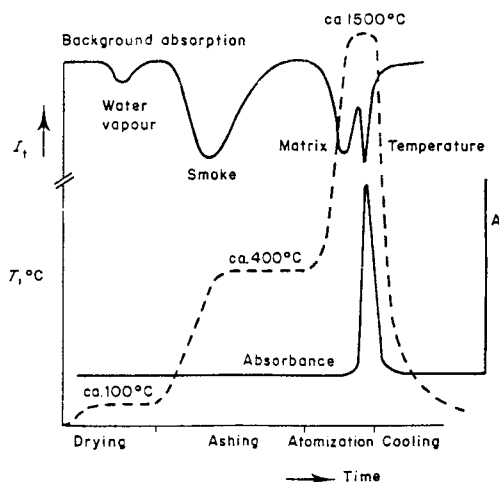
It is generally believed that the atomization mechanism for Pb involves reduction of the solid oxide on the graphite surface according to (2, p. 108).



Most commercial electrothermal atomizers based on the L'vov furnace as simplified by Massman (3) and undergo vigorous changes in tube temperature. The analyte atoms volatilized from the tube wall come into a cooler gas, so that molecular species that are not detected are formed. This leads to what is termed "matrix interferences." For example, equal concentrations of Pb^{2+} in a matrix of deionized water versus one of high chloride content would yield different absorbances. One way to remove this chloride matrix interference is to use a "matrix modifier." The addition of an ammonium salt to the chloride-containing matrix would cause volatilization of NH_4Cl with removal of chloride from the sample matrix.

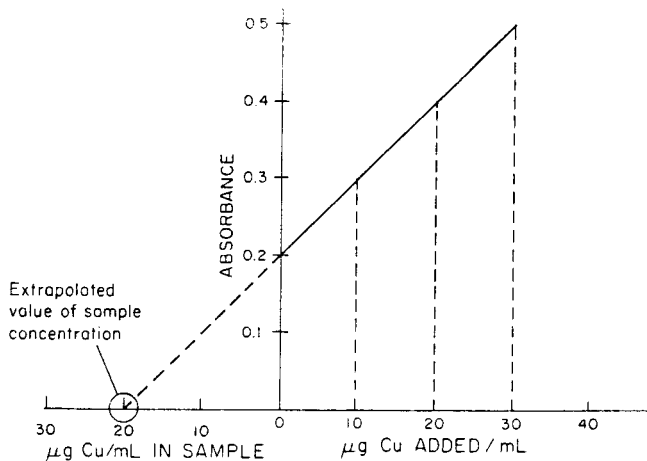
Each end of the furnace tube is connected to a high-current, programmable power supply through water-cooled contacts. The power supply controlling the furnace can be programmed to dry, ash, and atomize the sample at the appropriate temperatures. The temperature and duration of each of these steps can be controlled over a wide range. The optimization of the operating conditions is very important in developing methods using GFAA. A description of the three major temperature program changes known as "steps" is important in understanding GFAA operation. In the first step (drying), the solvent is evaporated at a temperature just above its boiling point. For aqueous solutions, the temperature is held at 110°C for about 30 s. In the second step (ashing), the temperature is raised to remove organic matter and as many volatile components from the sample matrix as possible

without analyte loss. The ashing temperature varies from 350°C to 1200°C and is maintained for about 45 s. The last step (atomization) occurs between 2000°C and 3000°C and lasts for just a few seconds. The element of interest is atomized and the absorption of the source radiation by the atomic vapor is measured. The furnace is then cleaned by heating the atomizer to the maximum temperature for a short period. Finally, the temperature is decreased to room temperature using water coolant and inert gas flow (argon). This process is depicted graphically below. Note that the graph includes the overall transmitted intensity I_t , the furnace temperature T , and the net absorption after background correction. It becomes important to have a means to subtract out this background. The Perkin-Elmer Model 3110 AA uses a deuterium background correction technique (4).



Of the three modes of calibration used in instrumental analysis, external, internal, and standard addition, the latter provides for the most accurate analytical results for samples which exhibit a matrix interference. The external mode of calibration is used to convert instrumental response to concentration when matrix interferences are not considered a factor. A series of standard solutions which contain the metal of interest are prepared from precise dilution of a certified standard stock solution. The calibration curve is obtained and a least squares regression is performed on the x, y data points. The best-fit line is used to establish the calibration curve. Samples which contain the metal at an unknown concentration level in a sample matrix nearly identical to that used to prepare the serial standards can be run and the data interpolated to give the concentration. In contrast, the standard addition mode of calibration requires that calibration and analysis

be performed on the sample itself. Standard addition can be used provided that (a) a linear relationship exists between the physical parameter being measured and the concentration of analyte, (b) the sensitivity of the method is not changed by the additions, and (c) the method blank can be corrected for (2, pp. 66–67). A typical standard addition calibration follows:



This exercise affords students an opportunity to operate a Perkin-Elmer Model 3110 GFAA with the objective of determining the concentration of Pb at ppb levels by calibrating the instrument using two of the three modes: external standard and standard addition. Following establishment of the working calibration curve, an instrument calibration verification (ICV) standard containing Pb will be prepared, run in triplicate, and the precision determined. At least one unknown sample will be provided for students to determine the concentration of Pb. Students are asked to bring in a sample of drinking water to determine the concentration of Pb once the GFAA has been successfully calibrated and the precision and accuracy for the ICV has been found to be acceptable.

Experimental

Preparation of Chemical Reagents

NOTE: ALL REAGENTS USED IN THIS ANALYTICAL METHOD CONTAIN HAZARDOUS CHEMICALS! WEAR APPROPRIATE EYE PROTECTION, GLOVES, AND PROTECTIVE ATTIRE. USE OF CONCENTRATED ACIDS AND BASES SHOULD BE DONE IN THE FUME HOOD.

Reagents Needed per Student or Group

- 1 40 ppb reference standard containing Pb in 1% spectrograde nitric acid
- 1 Blank reference standard in 1% spectrograde nitric acid
- 1 Matrix modifier
- 1 Unknown sample containing Pb; be sure to record the code
- 1 Drinking water from any source

Procedure

The setup and operation of the GFAA involves the following sequence of activities (5):

1. Install and align the hollow-cathode lamp (HCL)
2. Align the furnace in the spectrophotometer
3. Install and condition a new tube
4. Setup an element parameter file within the WinLab software
5. Align the autosampler
6. Place blanks, modifiers, and the standard in appropriate locations in the autosampler carousel
7. Run the calibration according to the programmable sequence within the WinLab software
8. Run any and all samples provided the calibration and ICV are acceptable

Using the WinLab[®] Software

You should find the WinLab (Perkin-Elmer) software used to control and to acquire GFAA data already downloaded. Retrieve and enter the software via the keyboard. The Pb HCL should turn on as well. A stable signal is necessary in order to continue. Proceed to autozero the detector, and if not satisfied, go to the “realign lamps” screen and adjust the physical position of the Pb HCL so as to bring the energy throughput near to that for the deuterium lamp. Set up a sequence to run a lab blank, a series of calibration standards for Pb, and one or more ICVs.

Evaluate the quality of the calibration and, if satisfactory, run the ICV under the “run samples.” Retrieve the weight/ID screen and choose an autosampler location and run in triplicate. If precision is acceptable, set up the weight/ID screen with the fortified Pb sample and any other samples of interest.

Preparation of the Stock Reference Pb Standard and Start of the Autosampler

To prepare the calibration standards for Pb, obtain the certified Pb stock standard, which should be 1000 ppm as Pb. Using an appropriate liquid syringe, take 40 μL of the 1000-ppm Pb stock and place this aliquot into a 10-mL volumetric flask already half-filled with 1% spectrograde nitric acid. Adjust to final volume using the 1% acid. Transfer this solution to a storage container and label “4 ppm Pb.” Take 100 μL of the 4-ppm Pb and place this aliquot into a clean 10-mL volumetric flask. Adjust to final volume and transfer to a clean storage container and label “40 ppb Pb.” Transfer about a 1-mL aliquot of the 40-ppb Pb reference standard to a plastic GFAA autosampler vial and place in location #38. Place a clean vial which is filled with 1% nitric in location #36. Place a clean vial which is filled with matrix modifier in location #37. The programming for the autosampler is found under “element parameters\calib”; retrieve this and write the entries into the table into your lab notebook.

Using the 40-ppb Pb standard and the WinLab software, implement an *external mode* of calibration. Retrieve or create a method title for this and conduct the calibration and quantitation. Evaluate whether the calibration is free from systematic error. If so, inject the ICV in triplicate. Record the code for the unknown and inject in triplicate. Run one or more drinking water samples.

Proceed on to the implementation of the *standard addition mode* of calibration using the 40-ppb Pb standard and the WinLab software. Retrieve the method and conduct the calibration and quantitation. Evaluate whether the calibration is free from systematic error. Inject the ICV in triplicate. Record the code for the unknown and inject in triplicate. Run one or more drinking water samples. Rerun one or more samples, provided the calibration is linear and the precision and accuracy for the ICV is acceptable.

For the Notebook

Include in your notebook the following: data from both modes of calibration for each of the two metals, calculate the confidence limits at 95% probability using the Student's *t*-statistics for the ICV for both calibration modes. Report on the concentration of Pb in the coded unknown provided to you. Report on the Pb concentration of any unknown drinking water sample that you analyzed. Two statistical computer programs RSD and LSQUARES and written in BASIC are available for your use on the laboratory PCs. These programs are found in Appendix C. To use these programs, first download “GWBASIC.exe” on the contemporary Windows-based

PCs. Proceed to calculate the means, standard deviations, relative standard deviations, and confidence intervals at a given probability. Calculate the relative error between the mean result for the ICV and the expected result for both types of calibration modes (i.e., external standard and standard addition). Comment on the effect of the external versus standard addition mode of GFAA calibration on the precision and accuracy in the ultratrace determination of Pb in drinking water.

Discuss why background correction is necessary in AA. Distinguish between physical and chemical interferences in AA. Explain what is meant by the term “Smith–Hieftje background correction” and discuss how it differs from the kind of correction employed in the Perkin–Elmer Model 3110 AA.

References

1. Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, SW 846, Revision 1, 1987, Method 7000, Atomic Absorption Methods.
2. Vandecasteele C, C Block. Modern Methods for Trace Element Determination. New York: John Wiley & Sons, 1993.
3. Massman H. Spectrochim Acta Part B 23B:215, 1986.
4. Skoog D, J Leary. Principles of Instrumental Analysis. 4th ed. Philadelphia: WB Sanders, 1992, pp 216–218.
5. Training Manual, Graphite Furnace Atomic Absorption with WinLab Software, Perkin-Elmer.

DETERMINATION OF THE DEGREE OF HARDNESS IN VARIOUS SOURCES OF GROUNDWATER USING FLAME ATOMIC ABSORPTION SPECTROSCOPY

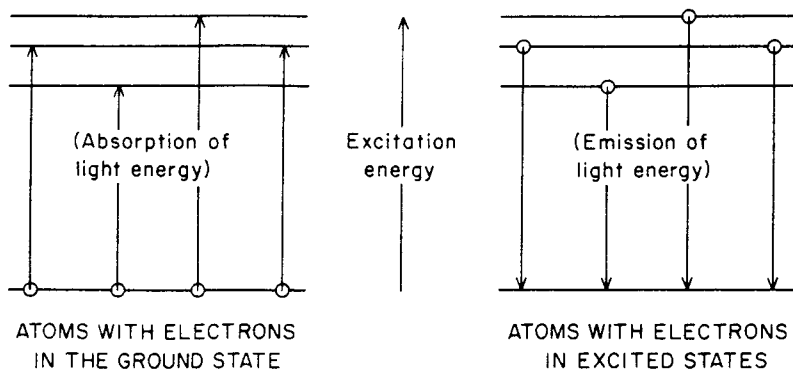
Background and Summary of Method

The extent to which groundwater has been rendered “hard” has been defined as the concentration of dissolved bicarbonates containing calcium and magnesium ions present in the sample. Hardness can be quantitatively measured by finding some way to measure these two alkaline-earth metal ions. The classical method which still finds widespread use is that of titration with EDTA. We are going to approach the problem by measuring the concentration of Ca^{2+} by flame atomic absorption spectroscopy (FAA) and by a mere change of hollow-cathode lamps, by measuring the concentration of Mg^{2+} . Several sources of groundwater will be obtained and the Ca/Mg content will be used to estimate the degree of water hardness.

Recall that an analytical method’s precision is a measure of the degree to which it can be reproduced or repeated and is evaluated by calculating the standard deviation for the method’s instrument calibration verification (ICV) standard following establishment of a single-point or multipoint cali-

bration. A method's accuracy is a measure of how close the results are to an established or authoritative value and is evaluated by calculating the percent relative error.

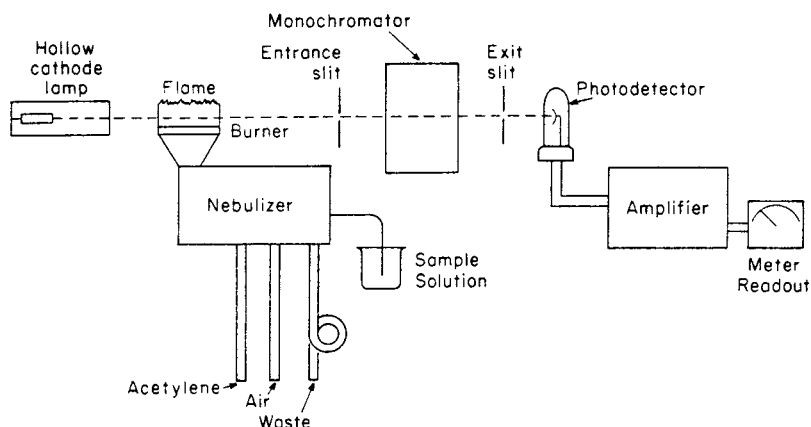
Flame atomic absorption spectrometry requires a means by which an aqueous solution containing metal ions can be aspirated into a reducing flame environment by which atomic Mg or Ca vapor is formed. Photons from the characteristic Mg emission of a hollow-cathode lamp (HCL) are absorbed by ground-state Mg atoms present in the approximately 2300°C air-acetylene flame. The amount of radiant energy absorbed as a function of concentration of an element in the flame is the basis of AA and follows Beer's law. In contrast to molecular absorption in solution, atomic spectra consist of lines and originate either from atomic absorption or atomic emission processes, which are depicted schematically below (1, Chap. 9):



Instrument detection limits (IDLs) for most metals by FIAA are in the low-ppm realm in contrast to graphite furnace AA (GFAA). The conventional premixed chamber-type nebulizer burner is common. The sample is drawn up through the capillary by the decreased pressure created by the expanding oxidant gas at the end of the capillary, and a spray of fine droplets is formed. The droplets are turbulently mixed with additional oxidant and fuel and pass into the burner head and the flame. Large droplets deposit and pass down the drain; 85–90% of the sample is discarded in this way. Figures 10-15 in Ref. 2 (pp. 216–218) provides a good schematic of the laminar flow burner.

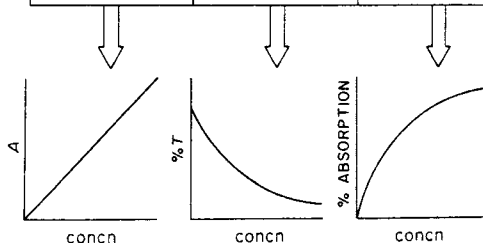
Flame atomic absorption spectrometry was developed in 1955 independently by Walsh in Australia (3) and by Alkemade and Milatz (4) in the Netherlands. Because electrons in quantized energy states for atoms of alkali metal elements can easily be raised to excited states (see above), flame emission spectroscopy is a more appropriate instrumental technique,

whereas plasma sources are needed for atoms of most other elements. Atomic absorption spectroscopy is unique in that it uses a flame to create the atomic vapor within which the absorption of radiation from a HCL can occur. This enables the quantitative determination of some 65 elements (1, p. 245), provided a line source can be used. The source of light for AA must produce a narrow band of adequate intensity and stability for prolonged periods of time. An ordinary monochromator is incapable of yielding a band of radiation as narrow as the peak width of an atomic absorption line. Hollow-cathode lamps satisfy these criteria (2, pp. 211–214). Refer to the following schematic:



Many models of atomic absorption spectrophotometers are in use in environmental testing laboratories today. Because of this, the type of readouts that one may get might differ. Older instruments most often used % absorption, whereas more contemporary instruments might read out in absorbance or % transmittance. The following schematic relates all three types of AA readouts (1, p. 247):

Absorbance	% Transmittance	% Absorption
0	100	0
0.045	90	10
0.097	80	20
0.155	70	30
0.229	60	40
0.301	50	50
0.398	40	60
0.523	30	70
0.699	20	80
1.00	10	90
∞	0	100



This exercise introduces students to trace metals analysis for determining the concentration of Ca and Mg using FIAA techniques. The Perkin-Elmer Model 3110 AA needs to be set up in the flame mode of operation. The FIAA will be calibrated and the calibration verified. Various sources of groundwater need to be obtained.

Experimental

Preparation of Chemical Reagents

NOTE: ALL REAGENTS USED IN THIS ANALYTICAL METHOD CONTAIN HAZARDOUS CHEMICALS! WEAR APPROPRIATE EYE PROTECTION, GLOVES, AND PROTECTIVE ATTIRE. USE OF CONCENTRATED ACIDS AND BASES SHOULD BE DONE IN THE FUME HOOD.

Chemicals/Reagents Needed per Student or Group

250 mL 1% HNO_3 (use spectrograde nitric acid only); if unavailable, prepare by placing 3.6 mL of concentrated HNO_3 into a 250-mL volumetric flask previously half-filled with distilled, deionized (DDI) water, adjust to the calibration mark with

DDI; transfer to a plastic storage bottle and label “1% HNO₃, spectrograde”

5 mL 1000 ppm Mg (certified stock solution)

5 mL 1000 ppm Ca (certified stock solution)

FIAA Operating Analytical Requirement (5)

Specification	Ca	Mg
Optimum range of concentration (minimum)	0.2	0.02
Optimum range of concentration (maximum)	7.0	0.05
Wavelength (nm)	422.7	285.2
Sensitivity (ppm)	0.08	0.007
Instrument detection limit (IDL) (ppm)	0.01	0.001

If a wavelength of 202 nm is used for Mg, a wider linear dynamic range is available (i.e., from 0 to 10 ppm). The sensitivity and IDL will be different.

Preparation of the Calibration Curve

As an illustration only, working calibration standards could be prepared as suggested below (this scheme assumes that the 1000 ppm certified stock reference standard solution has been carefully diluted to 3 ppm):

Standard No.	# mL 3 ppm	Final volume (mL) ^a	Conc. Mg (ppm)
0	0	50	0
1	4.15	50	0.25
2	12.5	50	0.75
3	25	50	1.5
ICV	10	50	0.6

^a Use 1% HNO₃ for all dilutions.

Procedure

The detailed instructions on how to set up and operate a FIAA using the Model 3110 AA will be made available in the laboratory (6). The burner–nebulizer attachment will need to be installed and the energy throughput optimized using either the Ca or Mg HCL. Proceed to aspirate the working calibration standards. An instrument calibration verification (ICV) standard should be prepared whose concentration should be approximately between the low and high standards. It should be sufficiently aspirated so that triplicate determinations of the absorbance from the same ICV solution can be

made. If the precision and accuracy for the calibration and ICV are found acceptable, then any and all available unknowns can be run. A “fortified sample” should be available which should contain both Ca and Mg. This sample may have to be diluted if the absorbance is found to significantly exceed the linear dynamic range of the instrument. Be sure to **record the name or the correct code** for each unknown sample. Establish the IDL experimentally for your instrument for each of the two metals. This is accomplished by obtaining seven replicate absorbance measurements of your blank standard.

For the Lab Notebook (No Report Necessary)

Conduct a determination of the IDL for each metal by measuring the signal due to a blank which is repeatedly aspirated into the flame seven times. Apply the principles discussed in Ref. 2 (pp. 7–8) and calculate the IDL assuming $k = 3$. Establish a least squares regression fit to the experimental calibration data points for both metals. Calculate the standard deviation in both the slope and y intercept for the calibration curve for both metals. Calculate the standard deviation in the measurement of the ICV for triplicate absorbance measurements. Use equations presented in Chapter 2 or obtain via a computer program. LSQUARES, a routine written in GW BASIC, is available on the laboratory PCs for your use. Access via C:\statisti from Windows File Manager. Calculate the confidence limits for the ICV for each metal. Calculate a percent error in the ICV for each metal. Report the concentrations for both Ca and Mg in the fortified sample and for one or more environmental samples. Comment on the differences in IDLs for Ca and Mg using FLAA.

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6. Model 3110 Atomic Absorption Spectrometer. User's Guide. Perkin-Elmer Corporation, 1993, Chaps. 3–9.

**DETERMINATION OF OIL AND GREASE AND OF TOTAL PETROLEUM HYDROCARBONS IN
WASTEWATER VIA REVERSED-PHASE SOLID-PHASE EXTRACTION TECHNIQUES
(RP-SPE) AND QUANTITATIVE FOURIER-TRANSFORM INFRARED
SPECTROSCOPY (FTIR)**

Background and Summary of Method

One of the most informative and straightforward analytical methods involves the quantitative analysis of environmental samples which have been contaminated with what can be collectively termed “oil and grease.” The term “oil and grease” refers to any and all hydrocarbons and includes lipids, high-molecular-weight fatty acids, triglycerides, higher olefinic hydrocarbons, alkanes, alkenes, monocyclic and polycyclic aromatics, and so forth. The total petroleum hydrocarbon (TPH) content of this oil and grease contamination can also be quantitatively determined by this method if the extracted sample is placed in contact with silica gel. The software that will be used with the FTIR spectrophotometer distinguishes between these two definitions. The absorption in the infrared region of the electromagnetic spectrum due to the presence of aliphatic and aromatic carbon to hydrogen-stretching vibrations occurs in the $3100\text{--}2900\text{-cm}^{-1}$ range. These concepts form the physical-chemical basis for the instrumental measurement.

The method is adapted from Method 5520 “Oil and Grease” in *Standard Methods for the Examination of Water and Wastewater* (1) and follows from EPA Method 413.2 (2). A recent revision of the procedures have appeared as EPA Method 1664 and calls for a replacement of the infrared absorption measurement by a gravimetric technique following distillation (3). The FTIR technique will be used here and we will **responsibly recover** the solvent whose waste disposal is the subject of current environmental concern and debate. Software developed at Perkin-Elmer Corporation during the late 1980s has been downloaded to the Model 1600 FTIR spectrophotometer and will be used to provide for the quantitative analysis (4). The original concept was first published by Gruenfeld at the EPA in the early 1970s (5).

An aqueous sample whose oil and grease content is to be determined is first acidified then extracted using 1,1,2-trichloro-1,2,2-trifluoroethane (TCTFE). Molecules of this solvent lack a C—H covalent bond and thus serves to provide an excellent background infrared spectrum because no absorption in the $3100\text{--}2900\text{-cm}^{-1}$ region is found. The extract is isolated from the aqueous matrix and an aliquot (a portion thereof) is transferred to a 1-cm (path length) quartz cuvette. The FTIR absorbance versus wavelength or wave number is graphically displayed on the screen. This absorbance, which is initially related to the concentration of “oil and grease” in standards, yields the concentration as “oil and grease” in the unknown

sample via interpolation of the least squares regression fit. The common concentration units used is mg “oil and grease”/100 mL of solution (oil and grease dissolved in TCTFE).

A soil/sediment or contaminated sludge with a very high solids content can be extracted via Soxhlet extraction techniques or more conveniently via ultrasonic probe sonication into a mixture of TCTFE and a carefully weighed amount of soil/sediment/sludge.

Experimental

Preparation of Chemical Reagents

NOTE: ALL REAGENTS USED IN THIS ANALYTICAL METHOD CONTAIN HAZARDOUS CHEMICALS! WEAR APPROPRIATE EYE PROTECTION, GLOVES, AND PROTECTIVE ATTIRE. USE OF CONCENTRATED ACIDS AND BASES SHOULD BE DONE IN THE FUME HOOD.

Reagents Needed per Student or Group of Students

- 0.1 g hexadecane, $C_{16}H_{34}$; alternatively, dodecane, $C_{12}H_{26}$, can be substituted
- 0.1 g iso-octane (2,2,4-trimethylpentane), $(CH_3)_3CCH_2CH(CH_3)_2$
- 0.1 g benzene, C_6H_6
- 250 mL TCTFE
- 100 mL MeOH (methanol); use as high a purity as is available
- 5 g silica gel, needed only if TPHs are to be determined in addition to oil and grease
- 5 g sodium sulfate anhydrous (Na_2SO_4)
- 20 mL 1 : 1 hydrochloric acid

Mix equal volumes of acid and deionized water. Remember always add acid to water!

Apparatus Needed per Group

- Vacmaster vacuum manifold to conduct solid-phase extraction (SPE) from International Sorbent Technology; the manifold enables 10 extractions to be performed at the same time
- Water trap and associated vacuum tubing to be used with the Vacmaster
- Suction vacuum pump connected to the water trap
- Accessories for use of the Vacmaster that includes receiving rack
- SPE cartridges packed with C_{18} -bonded silica
- 70 mL sample reservoirs
- 10 mL glass volumetric flasks

Procedure

Refer to the oil and grease analysis method in Ref. 1 and implement the appropriate procedure. For a wastewater sample, the procedure below has been developed in the Environmental Engineering Research Analytical Laboratories for your use. For wastewater samples, follow the procedure outlined below. If a percent recovery study is required, the procedure that immediately follows provides guidance for this study:

Percent Recovery Study

To isolate and recover TPHs from water by RP-SPE combined with quantitative FTIR:

- “Spike” approximately 200 mL of high-purity laboratory water that has been **acidified with 1 : 1 HCl** with approximately 2 mg of dodecane; note that most of the dodecane will “float” on top. This sample is called a *matrix spike* using terms first developed at the EPA. “Spike” a second 200-mL portion of **acidified** water with approximately 2 mg dodecane. This sample is called a *matrix spike duplicate*. Leave a third 200-mL portion of water unspiked and **acidify**. This sample is called a *method blank*.
- Set up three C₁₈ SPE cartridges and condition with MeOH.
- Pass the method blank, matrix spike, and matrix spike duplicate samples through the cartridges under vacuum.
- Remove water droplets with a Kim-Wipe; apply more vacuum to remove water from the sorbent.
- Elute off the cartridge with two 500-μL portions of TCTFE into a 1-mL volumetric as receiver; use glass syringe that is available near the SPE manifold.
- Transfer contents of the 1-mL receiver to the quartz cuvette and add exactly 1.0 mL of TCTFE using a glass pipette.
- Call previous calibration standards from disk on Model 1600 FTIR.
- Run each of the eluents from SPE. Transfer each eluent to a quartz cuvette and measure the absorbance. For the sample ID, enter any number. For the initial mass of sample, enter “200” and for the volume of sample after extraction enter “2.”

For the preparation of a control (i.e., a 100% recovered sample), weigh approximately 2 mg of dodecane into a 1-mL volumetric flask half-filled with TCTFE and adjust to the mark with TCTFE. Transfer to a quartz cuvette, add 1 mL of TCTFE, and measure the absorbance using the Model 1600 FTIR.

Probe Sonication: Liquid–Solid Extraction

It is first necessary to estimate to what extent the solid sample is laden with oil and grease. This minimizes the necessity to dilute the extract so that the absorbance will remain on-scale. This can be accomplished by taking 0.5-, 5-, and 15-g samples and using identical extraction volumes. Once the optimum sample weight has been estimated, proceed to the next step.

Calibration of the FTIR Spectrophotometer

Calibrate the Model 1600 FTIR (Perkin-Elmer) by first preparing a series of working calibration standards. The following table serves as a useful guide and yields concentrations that are compatible with the software which operates the instrument.

Prepare Blend A by obtaining a total weight of approximately 0.10 g for the pure form of the oil that is to be defined as the reference. For example, if hexadecane, iso-octane, and benzene are to be used and mixed, add approximately 0.033 g of each to obtain the desired weight. Transfer the oil to a clean, dry 10-mL volumetric flask. Add about 5 mL of TCTFE to dissolve the oil; then, adjust to the calibration mark. Transfer to a clean, dry glass vial with a Teflon/silicone septum and screw cap. Label this solution “Blend A, 1000 mg Oil and Grease/100 mL (in TCTFE)”; prepare Blends B through F according to the following table:

Blend	mL of Blend A	Extract volume ^a (mL)	Concentration (mg/100 mL)
F	0.01	10	1.0
E	0.1	10	10
D	0.2	10	20
C	0.3	10	30
B	0.4	10	40
ICV ^b	0.25	10	Unknown

^aUse 10-mL glass volumetric flasks and TCTFE to adjust to final volume.

^bICV = instrument calibration verification standard; run as if it were a sample; enter a “1” for sample weight and “1” for extract volume.

When you are ready to perform the calibration, retrieve under method “og & ph” (oil, grease, and petroleum hydrocarbons) and exercise one of the six options. It is important to obtain a fresh background by placing TCTFE into a clean 1-cm quartz cuvette. If the blank reveals a large absorbance, the **quartz cuvettes must be cleaned with detergent**. Contamination of the surface of the quartz cuvettes represents a major source of error with this method.

Isolation of Oil and Grease from Wastewater Samples

- Place approximately 200 mL of wastewater sample into a clean, dry 250-mL beaker using a graduated cylinder. **Record the volume of sample in your lab notebook.**
- Acidify to a pH of approximately 2 by adding sufficient 1 : 1 HCl.
- Set up the SPE vacuum manifold and condition the sorbent with MeOH; the sorbent surface should be wet with MeOH prior to passing the wastewater sample through the cartridge.
- Connect the 70-mL polyethylene sample reservoir to the cartridge via the adapter.
- Pass 200 mL of sample through under vacuum; observe that the sample actually flows through the SPE sorbent and watch for plugging.
- Remove the reservoir; remove the water droplets on the inner wall of the cartridge barrel.
- Elute with two 500- μ L of TCTFE directly into a 1.0-mL volumetric flask.
- Add enough anhydrous sodium sulfate, if necessary, to remove residual water in the eluent and stir.
- Transfer the contents of the volumetric to the quartz cuvette, add, with a pipette, 1 mL of additional TCFE, and measure the absorbance using the Model 1600 FTIR.
- The printer will give you a hardcopy output after a certain number of FTIR scans have been acquired. **Record all absorbance data in your laboratory notebook.** TRANSFER ALL WASTE TCTFE TO A PROPERLY LABELED WASTE RECEPTACLE FOR RECOVERY PURPOSES.
- If the absorbance is too large, make the appropriate dilution. Repeat the dilution step if necessary. TRANSFER ALL WASTE TCTFE TO A PROPERLY LABELED WASTE RECEPTACLE FOR RECOVERY PURPOSES.
- Prior to each day's FTIR measurements, the ICV standard should be measured and a log kept of its daily interpolated concentration. If the ICV value becomes a statistical outlier, rerun the calibration.

Calculations

The printout for a correctly measured TCTFE extract gives a direct value for the concentration of oil and grease in a solid sample provided the weight of sample is entered in grams and the volume of extract is entered in milliliters. Because we are measuring sample volume instead of weight, **substi-**

tute for the weight with 200 mL and use an extract volume of 2 mL. The calculation is based on the following:

$$\frac{(2 \text{ mL})(\text{mg oil and grease}/100 \text{ mL})(\text{DF})}{200 \text{ mL sample}} \frac{1000 \text{ mL}}{1 \text{ L}} = \frac{\text{mg}}{\text{L}}$$

where DF is the dilution factor. If no dilution, assume $\text{DF} = 1$. If a 1 : 25 dilution is made, DF would equal 25.

References

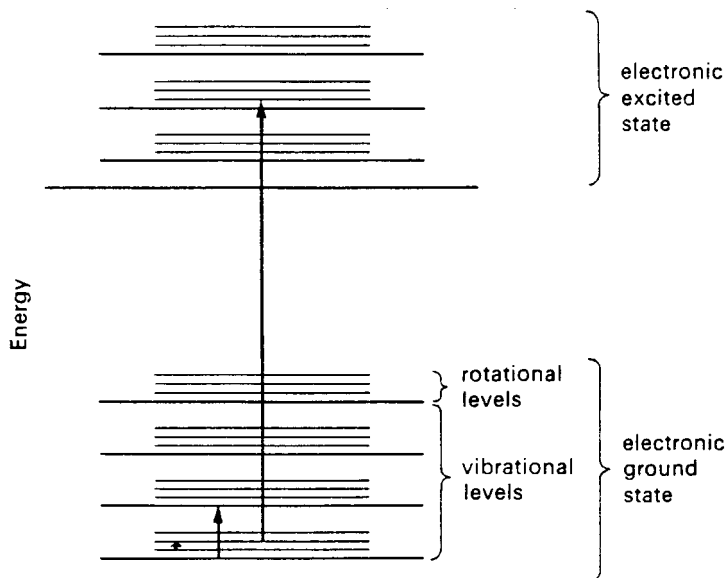
1. Eaton A, L Clesceri, A Greenberg (eds). Standard Methods for the Examination of Water and Wastewater. 19th ed. American Public Health Association, American Water Works Association, Water Environment Federation, 1995, pp 5–30–5–35.
2. Oil and Grease, Total Recoverable, Method 413.2 (Spectrophotometric, Infrared). Washington, DC: Environmental Protection Agency, 1978.
3. Method 1664: N-Hexane Extractable Material and Silica Gel Treated N-Hexane Extractable Material by Extraction and Gravimetry. Washington, DC: Office of Water Engineering and Analysis Division, 1994, EPA-821-B-94-004.
4. McGrattan B. 1600 Method Program Automating ASTM Standard Test Method for Oil and Grease and Petroleum Hydrocarbons in Water, Perkin-Elmer Infrared Bulletin 114.
5. Gruenfield M. Environ Sci Technol 7:636–639, 1973.

COMPARISON OF ULTRAVIOLET AND INFRARED ABSORPTION SPECTRA OF CHEMICALLY SIMILAR ORGANIC COMPOUNDS

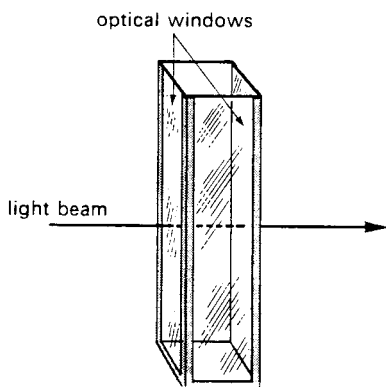
Background and Summary of Method

Because most organic compounds known to pollute the environment are colorless, it would be close to impossible to identify them and therefore to quantitate if only a visible spectrophotometer were available. Do not despair! The ultraviolet (UV) spectrophotometer enables the full UV region to be used and these organic contaminants can be identified. The UV region is defined to be those wavelengths from 190 to 400 nm. We know that the internal energies of atoms and molecules are quantized; that is, only certain discrete energy levels are possible and the atoms and molecules must exist at all times in one or the other of these allowed energy states. For absorption of radiation to occur, a fundamental requirement is that the energy of the photon absorbed must match exactly the energy difference between initial and final energy states within the atom or molecule. Consideration of atoms falls within the realm of atomic absorption and atomic emission, whereas molecular absorption involves transitions between electronic (ultraviolet–

visible absorption), vibrational (mid-infrared absorption), and rotational (microwave absorption). The following diagram depicts quantized energy states for organic molecules which are dissolved in a solvent (1):



The molecular absorption phenomenon can only be accurately measured provided that the ratio of transmitted intensity, I , of the UV radiation to that of the incident intensity, I_0 , is due to the presence of the dissolved solute and not to scattering of the incident beam. If the optical windows (see below) absorb UV radiation, then the absorbance which is related to the logarithm of the ratio I/I_0 would cause an increase in sample absorbance; hence, this would lead to an erroneous result. The student will encounter two types of optical window material. One consists of glass and is said to have a UV cutoff (UV wavelengths below this would absorb) of 300 nm (near UV) and the other consists of quartz with a UV cutoff of 190 nm. The rectangular cuvette is depicted as follows:



UV-Vis Absorption Spectroscopy

The light source for molecular absorption spectroscopy in the visible and UV regions (i.e., 750 nm down to 350 nm) is a tungsten filament lamp which radiates as a blackbody at about 2800 K. Below 350 nm, the hydrogen or deuterium gas discharge lamp is preferred. The *reciprocal linear dispersion* (refer to Ref. 3 or other equivalent text for explanation) is around 1 nm/mm for diffraction grating spectrophotometers and between 0.5 and 5 nm/mm for prism spectrophotometers (*note*: dispersion depends on wavelength for prisms). Air absorbs UV light of wavelengths shorter than about 180 nm, so studies at wavelengths shorter than 180 nm require the use of an evacuated spectrometer. This region is thus termed the vacuum ultraviolet (2).

The role of the solvent becomes critical in obtaining accurate UV absorption spectra. A solvent considered suitable for use in the UV-vis region must itself exhibit a low absorbance as well as dissolve the solute whose spectrum is sought experimentally. Fortunately, most common solvents are highly transparent to visible light, but all begin to absorb at some wavelength in the UV. It is not essential that the solvent have 100% transmittance, although it is desirable that as large a fraction as possible of the incident radiant energy be available for absorption by the solute. The following table lists the approximate absorption "cutoffs" for several widely used solvents. The cutoff defines a practical short-wavelength limit for the useful range of a solvent. Below this wavelength, the absorbance of the solvent if placed in a 1-cm cell exceeds 1.0 absorbance units full scale (AUFS).

Solvent	Wavelength (nm)	Solvent	Wavelength (nm)
Water	200	Dichloromethane	230
Hexane	200	Chloroform	245
Heptane	200	Carbon tetrachloride	265
Cyclohexane	210	Dimethyl sulfoxide	265
Methanol	210	Dimethylformamide	270
Ethanol	210	Benzene	275
Acetonitrile	190	Pyridine	300
Dioxane	215	Acetone	325

Different solutes exhibit different UV absorptivities. Recall that what is measured in a spectrophotometer is absorbance, and absorbance A is related to absorptivity ε via

$$A = \varepsilon bc$$

Two chemically different solutions which contain solutes at identical concentrations in a suitable solvent using the same cuvette would not be expected to have the same absorbance due to differences in absorptivity (earlier terms included molar absorptivity and extinction coefficient). If logarithms are taken on both sides of the above equation, we have

$$\log A = \log \varepsilon + \log bc$$

Because the $\log bc$ term is independent of wavelength, $\log A$ will vary only as a function of absorptivity. Thus, a plot of $\log A$ versus spectrophotometer wavelength setting will be the same even though concentrations and path lengths for individual samples may differ. In this way, a comparison of the different curves can be made.

Mid-Infrared Absorption Spectroscopy

The mid-infrared region of the electromagnetic spectrum starts on the higher energy at $2.5\ \mu\text{m}$ ($4000\ \text{cm}^{-1}$) and ends on the lower energy at $6\ \mu\text{m}$ ($650\ \text{cm}^{-1}$). Dispersive-type infrared absorption spectrometers have given way to Fourier-transform infrared spectrometers (FTIR). Chapter 12 of Ref. 3, or other equivalent text, should be reviewed to better understand the principles underlying infrared spectroscopy.

Experimental

This exercise affords students an opportunity to measure actual UV and FTIR absorption spectra of several organic solutes while comparing overall differences in UV and FTIR spectra for chemically similar and dissimilar organic solutes. The laboratory experiment focuses on the influence of delocalization of electron density and the nature of UV absorption spectra. The experiment also focuses on the relationship of organic functional group analysis and FTIR absorption spectra. A review of an appropriate text which reviews the theory of UV-vis and FTIR absorption spectroscopy and molecular structure gives an appreciation of what will be observed in the laboratory (3).

Two sets of illustrative solutions are to be studied in this exercise. The first set consists of organosulfonates and enables a comparison of UV absorption spectra of alkane versus aromatic sulfonates which are dissolved in water. The second set consists of two representative organic esters which differ in their carbon backbone. The student is to engage both the UV spectrophotometer and the FTIR spectrometer in accomplishing the experimental aspects of this exercise.

Items/Accessories Needed per Student or Group

- 1 Pair of matching rectangular quartz cuvettes which transmit between 200 and 350 nm
- 1 Polyethylene (PE) disposable IR card (Type 61, 3M)
- 1 Polytetrafluorethylene (PTFE) disposable IR card (Type 62, 3M)
- 1 Model 160-UV (Shimadzu) UV-vis scanning absorption spectrophotometer or other equivalent scanning instrument
- 1 Model 1600 Fourier-transform infrared spectrometer (Perkin-Elmer) or other equivalent instrument.

Preparation of Chemical Reagents

NOTE: ALL REAGENTS USED IN THIS ANALYTICAL METHOD CONTAIN HAZARDOUS CHEMICALS! WEAR APPROPRIATE EYE PROTECTION, GLOVES, AND PROTECTIVE ATTIRE. USE OF CONCENTRATED ACIDS AND BASES SHOULD BE DONE IN THE FUME HOOD.

Procedure to Obtain UV Absorption Spectra for Two Sets of Chemically Similar Organic Compounds: (1) An Alkane Sulfonate Versus Alkyl Sulfate and (2) Two Esters with Different Carbon Backbones

Prepare two aqueous solutions which contain approximately 1000 ppm each of 1-octanesulfonic acid or sodium dodecyl sulfate (use whichever is available) **and** *p*-toluenesulfonic acid dissolved in distilled, deionized water (DDI). Transfer a portion of this solution into a quartz, rectangular cuvette and record the UV absorption spectrum for both organic compounds. Prepare a 100-ppm solution that contains methyl methacrylate and a 100-ppm solution that contains ethyl glycolate. You will need to use the wavelength cutoff guide to choose a suitable solvent because these organic compounds do not dissolve to any great extent in water. Record the UV absorption spectrum between 200 and 350 nm. Compare the spectra when a solvent is placed in the reference beam of the dual-beam instrument.

Procedure to Obtain FTIR Absorption (Transmission) Spectra for Various Organic Compounds

Disks which are partially to fully transparent in the infrared are available to easily prepare samples. An aqueous solution containing the anionic surfactants can be deposited directly onto either the PTFE or PE disk. Allow sufficient time for the solvent to evaporate off of the disk. Conduct a survey scan and take 16 FTIR scans. Plot the spectra. Consult the staff for assistance with the Model 1600 FTIR (Perkin-Elmer). FTIR absorption (transmission) spectra for both sulfonate surfactants and for both esters can be obtained using this technique (4).

For the Report

This exercise has introduced selected principles of molecular UV and infrared spectroscopy for qualitative chemical analysis. Interpret the significance of these spectra in terms of molecular structure. You should have obtained hardcopy printouts of UV and FTIR spectra. Please include all relevant spectra in your report.

Suppose that a client sent to you a mixture that was prepared from the ethylbenzene and styrene solutions that you used in the lab. Would benzene be a suitable solvent to use in obtaining UV spectra for ethylbenzene and styrene? Discuss how you would design a quantitative analysis to determine the concentration of ethylbenzene and of styrene in the client's sample. Assume that you have available pure ethylbenzene and pure styrene (chemist's refer to these as neat forms of the liquids).

Experimental

This exercise introduces many of the techniques that are required to identify and quantitate an environmental pollutant while facilitating an understanding of the relationship between chemical principles and instrumental analysis

Preparation of Chemical Reagents

NOTE: ALL REAGENTS USED IN THIS ANALYTICAL METHOD CONTAIN HAZARDOUS CHEMICALS! WEAR APPROPRIATE EYE PROTECTION, GLOVES, AND PROTECTIVE ATTIRE. USE OF CONCENTRATED ACIDS AND BASES SHOULD BE DONE IN THE FUME HOOD.

Methylene Blue (MB)

Dissolve 0.05 g MB in 50 mL of distilled, deionized water (DDI).

3M Sulfuric Acid

Add 16.7 mL concentrated sulfuric acid to a 100-mL volumetric flask half-filled with DDI. Adjust to the calibration mark with DDI.

To Prepare a 0.5M Sulfuric Acid Solution

Add approximately 4 mL of the 3M sulfuric acid to a 25-mL volumetric flask half-filled with DDI, then adjust to the final volume. Transfer to a storage vial and label.

To Prepare a 0.1M Sodium Hydroxide Solution

Weigh approximately 0.1 g of NaOH pellets into a 50-mL beaker which contains approximately 20 mL of DDI. Dissolve with stirring. Transfer the contents of the beaker to a 25-mL volumetric flask and adjust to the final volume. Transfer to a vial and label.

To Prepare the Wash Solution

To a 1000-mL volumetric flask, half-filled with DDI, add 41 mL of 3M sulfuric acid to 5 g $\text{Na}_2\text{HPO}_4 \cdot \text{H}_2\text{O}$. Adjust to the mark with DDI.

To Prepare the MB Reagent

To a 500-mL volumetric flask half-filled with DDI, add the following:

15 mL of MB

20.5 mL of 3M sulfuric acid

25 g of $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$

Shake until dissolved. Then, adjust to the calibration mark with DDI. Transfer contents of volumetric flask to a glass storage bottle and label “MB Reagent.”

Operation and Calibration of the Orion SA 720A pH Meter

The pH meter must be set up and calibrated with two buffers. Use buffer solutions whose pH value are 7 and 10, as these buffers have pH values near required in this method. Refer to the detailed instructions available at your workbench.

Preparation of the 100-ppm Surfactant Stock Solution and General Comments on Standards

Dissolve approximately 0.01 g of sodium lauryl sulfate in between 50 and 10 mL of MeOH in a 50-mL beaker. Transfer the contents of the beaker to a 10-mL volumetric flask and adjust to the mark with MeOH. This yields a stock solution whose concentration is 1000 ppm. Transfer 1 mL using a glass pipette and pipette pump to a 10-mL volumetric flask. Adjust to the mark with DDI. This yields a primary dilution reference standard whose concentration is 100 ppm.

It becomes important to know the chemical nature of the particular surfactant to be used to prepare the stock standard for construction of the calibration table (Table 5.2). So that the number of parts per million can be related to the concentration of anionic sulfonate actually taken and extracted as an ion pair. For example, for *p*-toluenesulfonic acid, 100 mg as the *p*-toluenesulfonate ion is only 100.6 mg as the acid; however, 100 mg as the *p*-toluenesulfonate ion is 113.4 mg as its sodium salt. A stock solution is stable for no more than 1 week. Working solutions such as the 100-ppm

TABLE 5.2 Calibration Table

Sample	μL of 100 ppm surfactant	μg of surfactant added	mL MB reagent	mL DDI	mL CH_2Cl_2 (total)	Conc. original sample (ppm)
Blank	0	0	2.5	10	10	0
Std. 1	100	10	2.5	10	10	1.0
Std. 2	250	25	2.5	10	10	2.5
Std. 3	500	50	2.5	10	10	5.0
Std. 4	1000	100	2.5	10	10	10
ICV	400	40	2.5	10	10	4.0

surfactant should be prepared fresh daily. Keep in mind that approximately **0.1 g** of any pure solid dissolved in enough solvent to prepare **10 mL** of solution yields a standard whose concentration is approximately **10,000 ppm**.

Procedure for μ LLE

1. Using a clean 25-mL graduated cylinder, place 10 mL of an aqueous sample whose anionic surfactant concentration is to be determined into a 50-mL beaker. The aqueous sample could be a blank (i.e., a sample with all reagents added except for the analyte of interest), calibration standard, ICV, fortified (i.e., spiked) sample, or an actual unknown wastewater effluent sample.
2. Neutralize the sample to a pH between 7 and 8 by dropwise addition of either 1M NaOH or 0.5M H₂SO₄.
3. Add 2.5 mL of the MB reagent and swirl; then, transfer the contents of the beaker to a 30-mL glass separatory funnel. **Be sure the stopcock is closed.**
4. Add 2 mL of methylene chloride (dichloromethane) or equivalent solvent. Because methylene chloride is much denser than water, it will comprise the lower layer after the two phases separate.
5. Stopper the separatory funnel with the ground-glass top, invert the funnel, and shake. Be sure to vent the vapor. This should be done in the fume hood.
6. Withdraw the lower layer into a second clean beaker.
7. Extract the remaining aqueous phase twice more with 2-mL portions of methylene chloride. Then, combine the methylene chloride extracts. Approximately 6 mL of organic solvent should be obtained at this point.
8. Wash the combine extracts with Wash Solution. To do this, transfer the 6 mL of extract (MeCl₂ + dissolved ion pair) to a clean 30-mL separatory funnel. Add approximately 10 mL of Wash Solution, shake, allow time for the two phases to separate, then remove the lower layer directly into a clean 50 mL beaker.
9. Transfer the washed extract (approximately 6 mL) to a 10-mL volumetric flask and adjust to a final volume.
10. Transfer a portion of the 10 mL methylene chloride extract to a standard spectrophotometric curvette and measure the absorbance against methylene chloride as a blank in the reference

cell at 652 nm. Record and repeat steps 1 through 10 for all standards and samples.

11. *Discard the waste extract into the hazardous waste receptacle located in the laboratory.*

For the Report (a Written Laboratory Report Due on this Experiment)

Use a spreadsheet program such as EXCEL or equivalent to construct a four-point calibration plot. The plot should show absorbance values on the ordinate and concentration of surfactant in ppm on the abscissa. Calibration data and the least squares regression plotted in EXCEL for an external standard mode (Fig. 5.3) and the standard additions mode (Fig. 5.4) for the quantitative analysis of anionic surfactants using the methylene blue colorimetric method are shown. Use a least squares regression to fit the experimental points and calculate the correlation coefficient. Calculate and report on the percent error and the confidence interval at 95% probability for the ICV. A computer program written in BASIC is available for you to use to obtain the statistical results (refer to the program listing in Appendix C). Report on the concentrations of any unknown environmental samples to which this method was applied. Discuss what you learned from this environmental analysis method drawing on your previous experience with spectrophotometric methods. Comment on the precision and accuracy afforded by the analytical method.

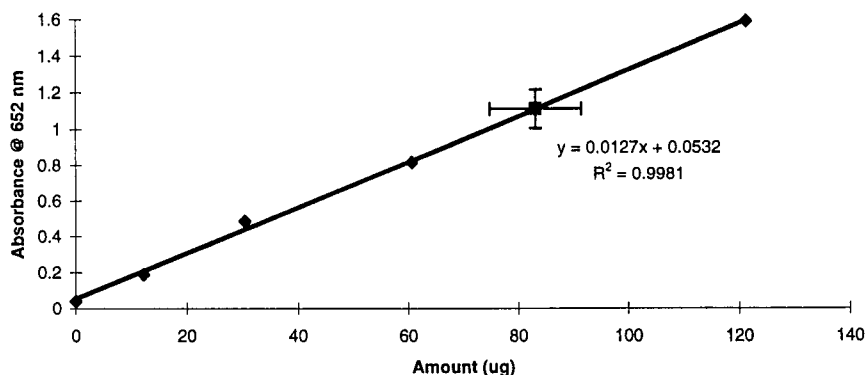


FIGURE 5.3 Calibration curve of anionic surfactants paired with methylene blue dye. The error bars represent the standard deviation in ICVs. ♦: standards; ■: ICV; linear regression on standards.

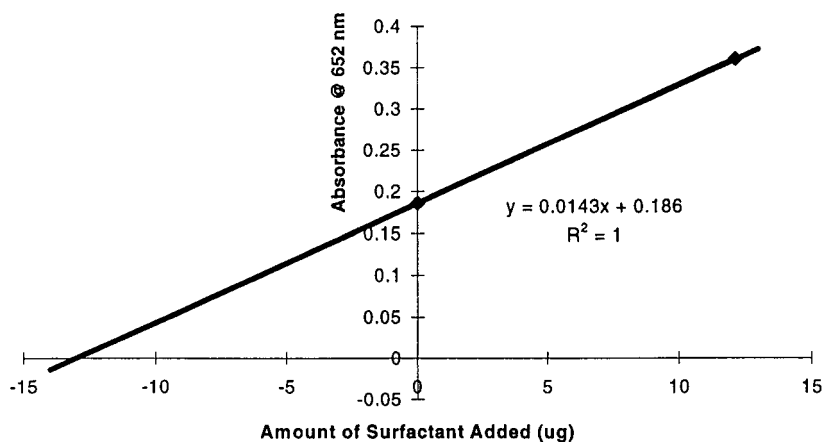


FIGURE 5.4 The graphical technique showing standard addition. The equation is for the linear regressed line through the points for the spiked sample and for the average of the triplicate runs of the unspiked samples.

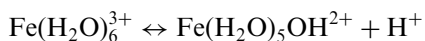
Reference

Adapted and modified from Schmitt TM. Analysis of Surfactants. Surface Science Series, Vol. 40. New York: Marcel Dekker Inc., 1992, pp 290–292.

VISIBLE SPECTROPHOTOMETRIC DETERMINATION OF TRACE LEVELS OF IRON IN GROUNDWATER

Background and Summary of Method

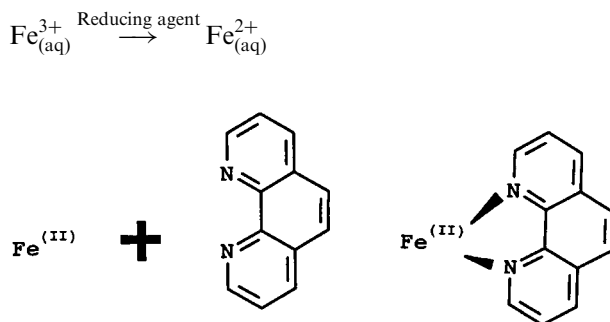
Iron (Fe), a metal in great abundance in the earth, is a common contaminant in groundwaters in its oxidized forms, ferric ion or iron(III) and ferrous ion or iron(II). Common rust consists of ferric oxides and persists in groundwaters as either solublized or as particulate matter. For environmental analytical purposes, one must distinguish between total Fe and dissolved Fe that could be present in groundwaters. It is also of interest to determine the degree of metal speciation [i.e., the concentration of Fe(III) to that of Fe(II)]. The hexaquo Fe(III) itself behaves as a weak acid and ionizes in water according to



thus contributing to groundwater acidity. Pure solutions of salts which contain the hexaquo Fe(III) are distinctly acidic, having a pH from about

2 to 4 depending on concentration (1). If either dissolved Fe or Fe(II) is to be determined, the sample must be analyzed as soon as possible after collection. If only total Fe is to be determined, the sample should be immediately acidified with hydrochloric acid (2).

Fe(II), once formed by reduction, forms an intensely colored complex ion with 1,10-phenanthroline according to the following reactions (3):



Only the Fe(II) oxidation state for iron forms the colored complex. Hence, this selectivity provides the basis for quantitatively determining the ferric/ferrous ratio that characterizes the dissolved Fe portion of the iron analysis. Several organic compounds that are readily soluble in water and easily reduce Fe(III) to Fe(II) are available and include hydroquinone, ascorbic acid, and hydroxylamine hydrochloride, among others.

This exercise affords an opportunity to introduce good laboratory practices when conducting an analysis using a spectrophotometer. Each student is given at least one unknown groundwater sample with which to measure the concentration of Fe(III) and the concentration of Fe(II).

You will need to consider how both oxidation states of iron can be quantitatively determined using the complexation with 1,10-phenanthroline method only. For a given volume of groundwater, the amount of Fe(III) and the amount of Fe(II) should approximate the amount of total Fe found independently by flame atomic absorption spectrophotometry (FAA).

Experimental

Volumetric Glassware Needed per Student

- 1 volumetric flask (100 mL)
- 1 pipette (5 mL)
- 1 pipette (10 mL)
- 1 volumetric flask (500 mL)
- 1 10 mL pipette calibrated in 1/10-mL increments (10 mL)

Gravity Filtration Setup

- 1 glass funnel and standard circular filter paper

Chemical Reagents Needed per Student or Group

NOTE: ALL REAGENTS USED IN THIS ANALYTICAL METHOD CONTAIN HAZARDOUS CHEMICALS! WEAR APPROPRIATE EYE PROTECTION, GLOVES, AND PROTECTIVE ATTIRE. USE OF CONCENTRATED ACIDS AND BASES SHOULD BE DONE IN THE FUME HOOD.

15 mL of 1.0% hydroxylamine hydrochloride; dissolve 10 g in every 100 mL of solution.

15 mL of 0.1% 1,10-phenanthroline; dissolve 0.1 g in enough acetone until completely solubilized, then add DDI for every 100 mL of solution.

5 mL of concentrated hydrochloric acid, HCl.

0.5 g of ferrous ammonium sulfate, $\text{Fe}_2(\text{NH}_4)_2(\text{SO}_4)_3$; dissolve 0.35 g in a 500-mL volumetric flask half-filled with DDI, then add 5 mL of concentrated HCl and adjust to the calibration mark with DDI.

This gives a solution which is 100 ppm as Fe.

100 mL of saturated sodium acetate solution; dissolve enough NaOAc until crystal formation is observed.

Spectrophotometer

A stand-alone UV-vis spectrophotometer such as a GENESYS[®] (Spectronic Instruments) or equivalent is suitable.

An atomic absorption spectrophotometer and/or an inductively coupled plasma spectrophotometer should be available if the instructor chooses to include these instruments in this exercise.

Procedure

1. Turn on the spectrophotometer and allow at least a 15-min warmup time. Set the wavelength to 508 nm.
2. Prepare the calibration standards by pipetting 0- (this is called the reagent blank), 1-, 2-, 3-, 4-, and 5-mL aliquots (portion thereof) of the 100 ppm Fe stock solution into a 100-mL volumetric flask. Also prepare an instrument calibration verification (ICV) standard by pipetting 2.5 mL of the 100-ppm Fe stock solution into a 100-mL volumetric flask. Measure the ICV's absorbance after developing the color below in triplicate. The ICV is used to evaluate the precision and accuracy of any instrumental method via interpolation of the calibration curve and is essential to maintain-

ing good quality control. **Hint:** To minimize contamination due to carryover when using only one volumetric, prepare standards from low to high concentration.

Standard No.	mL 100 ppm Fe	V (total)	Conc. (ppm)
Blank	0	—	0
1	1.0	100	1.0
2	2.0	100	2.0
3	3.0	100	3.0
4	4.0	100	4.0
5	5.0	100	5.0
ICV	2.5	100	2.5

3. To 20 mL of each reference standard solution, add 10 mL of saturated NaOAc and 10 mL of 1% hydroxylamine hydrochloride. Wait 5 min and add 10 mL of the 0.1% 1,10-phenanthroline solution. Allow 10 min, then dilute to the calibration mark of the 100-mL volumetric beaker with DDI.
4. Carefully filter approximately 35 mL of the unknown groundwater sample if necessary. Pipette 20 mL of sample and place into a clean 100-mL volumetric beaker. Add reagents as done previously for the calibration standard preparation and adjust to the final volume with DDI.
5. Set the zero control and 100% transmittance controls according to the operating manual instructions using DDI. **Record transmittance values for the blank calibration standards, ICV measured in triplicate, and unknown sample.**

Determination of Total Fe by FIAA or ICP–AES

You may have an opportunity to use either FIAA or ICP–AES to quantitatively measure the total iron in the same groundwater samples that were used above. The same set of calibration standards that you prepared can be used and directly aspirated into either the flame or the plasma. If you choose FIAA, you may need to install an Fe hollow-cathode lamp.

For the Notebook

Use a spreadsheet program such as EXCEL or its equivalent to construct a six-point calibration plot. The plot should show absorbance values on the ordinate and concentration as ppm Fe on the abscissa. Use a least squares regression to fit the experimental points and calculate the correlation coefficient. Calculate the percent error and the confidence interval at 95% prob-

ability for the ICV. Interpolate the calibration plot and obtain a concentration for Fe(III) and Fe(II) from the visible spectrophotometer. Obtain the concentration, C , for Fe(total) from the AA spectrophotometer. From these results, compare the calculated $C_{\text{Fe(II)}}/C_{\text{Fe(III)}}$ ratio from the colorimetric method against the AA method (i.e., conduct a mass balance). A computer program written in BASIC is available with which to use to obtain the statistical results. Refer to Appendix C for the statistical treatment.

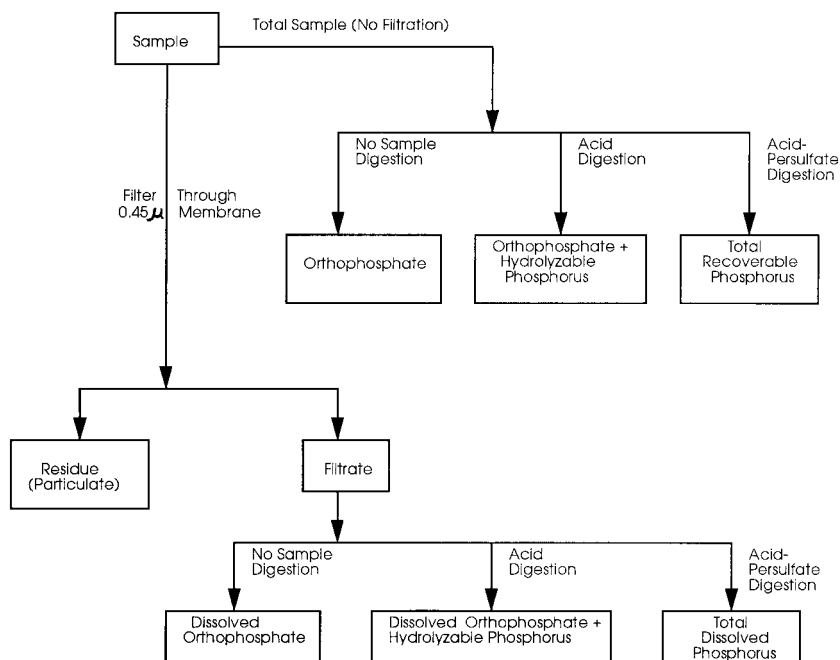
References

1. Lingane J. Analytical Chemistry of Selected Metallic Elements. New York: Reinhold, 1966, pp 56–57.
2. Annual Book of ASTM Standards, Part 31, Water, 1980. Philadelphia: American Society of Testing Materials, 1980, pp 438–442.
3. Pietryzk D, C Frank. Analytical Chemistry. 2nd ed. New York: Academic Press, 1979, pp 656–657.

SPECTROPHOTOMETRIC DETERMINATION OF PHOSPHORUS IN EUTROPHICATED SURFACE WATER

Background and Summary of Method

The persistence of phosphates in lakes, rivers, and streams due to domestic and industrial pollution has led to elevated levels and is regarded as largely responsible for lake eutrophication. This was an even more serious problem up until some 20 years ago, prior to the ban on phosphate containing detergents. In considering an environmental sample, one must distinguish between several chemical forms which contain the element phosphorus. Separation into dissolved and total recoverable phosphorus depends on filtration through a 0.45- μm membrane filter. Dissolved forms of phosphorus (P) include meta-, pyro-, and tripolyphosphates. The visible spectrophotometric procedure requires that all forms of P be chemically converted to the water-soluble orthophosphate ion, PO_4^{3-} . Hence, an acid hydrolysis step must be included in the method, which should account for all “hydrolyzable P.” A more rigorous conversion is necessary to include organophosphorous compounds and involves an acid–persulfate digestion of the sample. The following flowchart summarizes the analytical scheme to differentiate the various chemical forms of phosphorus (1):



Orthophosphate forms a complex with the molybdate ion in the presence of a reducing agent such as hydrazine sulfate, amino-naphthol-sulfonic acid, tin(II) chloride, or ascorbic acid, which is commonly called “heteropoly blue” and has a molecular formula: $\text{H}_3\text{PO}_4(\text{MoO}_3)_{12}$ (2). There are two wavelengths that can be used: one between 625 and 650 nm and a more sensitive one at 830 nm. The Spectronic 21 DUV is capable of measuring up to 1000 nm and the Spectronic 20 can go only up to 600 nm with the standard phototube. An infrared phototube is necessary to widen the wavelength of this instrument.

This exercise provides an opportunity to reinforce the principles of spectrophotometry and its relationship to environmental analysis. To minimize laboratory time, the acid hydrolysis step will be eliminated and, thus, only dissolved orthophosphate will be measured. In addition to determining the phosphorus content of a surface-water sample, each student will be given an unknown sample by the instructor whose phosphorus concentration has been previously determined by anionic ion chromatography.

Experimental

Preparation of Chemical Reagents

NOTE: ALL REAGENTS USED IN THIS ANALYTICAL METHOD CONTAIN HAZARDOUS CHEMICALS! WEAR APPROPRIATE EYE PROTECTION, GLOVES, AND PROTECTIVE ATTIRE. USE OF CONCENTRATED ACIDS AND BASES SHOULD BE DONE IN THE FUME HOOD.

5M Sulfuric Acid

Use an appropriate sized graduated cylinder and add 70.25 mL of concentrated sulfuric acid to a 250-mL volumetric flask which has been previously half-filled with distilled, deionized water (DDI). This solution will release heat. Allow to cool; then, fill to the calibration mark with DDI. Label as appropriate. The unused 5M sulfuric could be saved, diluted, and used in the exercise “Determination of Anionic Surfactants . . .”.

Molybdate Reagent

Dissolve 12.5 g of ammonium molybdate in approximately 100 mL DDI in a 500-mL volumetric flask. Add 150 mL of 5M sulfuric acid. Fill to the calibration mark with DDI. Label as appropriate.

1% Ascorbic Acid

Dissolve 1 g ascorbic acid into a 100-mL volumetric flask half-filled with DDI. Adjust to the calibration mark with DDI. Label as appropriate.

Preparation of Stock Phosphorus Standards (1)

Dissolve 0.2197 g potassium dihydrogen phosphate, KH_2PO_4 , that has been dried for one hour at 104°C in approximately 200 mL DDI in an appropriately sized beaker. You will need use of a correctly calibrated analytical balance. After dissolution is complete, transfer the solution to a 1 L volumetric flask and adjust to a final volume with DDI. Label as “1.0 mL = 0.05 mg P”. Carefully pipet 50 mL of the stock solution into a 1 L volumetric flask half-filled with DDI. Adjust to the calibration mark with DDI. Label as “1.0 mL = 0.0025 mg P”.

Preparation of the Calibration Standards According to the Following Table:

Sample	mL std. P (1 mL = 0.0025 mg)	mL Molybdate reagent	mL Ascorbic acid reagent	V_T (mL)	Conc. (ppm)
Blank	0	5	3	50	0
Std. 1	1	5	3	50	0.05
Std. 2	2	5	3	50	0.10
Std. 3	4	5	3	50	0.20
Std. 4	7	5	3	50	0.35
Std. 5	10	5	3	50	0.50
ICV	5	5	3	50	0.25 (expect)

Procedure (3)

A 5-mL aliquot of sample is taken and transferred to a 50-mL volumetric flask. Add 5 mL of the molybdate reagent and 3 mL of the reducing agent. The mixture is diluted to volume, and after waiting 6 min for color development (time for development for standards and unknown should be the same), the absorbance is determined at 830 nm using the Spectronic 21DUV or equivalent spectrophotometer. The ICV should be prepared in triplicate and its absorbance measured three times. Follow good laboratory practices (GLP) as was discussed in Chapter 2.

For the Notebook

Use a spreadsheet program such as EXCEL or its equivalent to construct a five-point calibration plot. The plot should show absorbance values on the ordinate and concentration as ppm P on the abscissa. Use a least squares regression to fit the experimental points and calculate the correlation coefficient. Calculate the percent error and the confidence interval at 95% probability for the ICV. Report on the unknown ppm P and estimate the confidence interval at 95% probability for both the surface-water sample and the sample given to you by the instructor; be sure to include the code for this sample. Review this method in a resource such as *Standard Methods for the Examination of Water and Wastewater* or other sources of the colorimetric determination of phosphorus and discuss the effect of matrix interferences on the precision and accuracy for determining P in environmental samples using the visible spectrophotometric method. A computer program written in BASIC (refer to Appendix C) is available with which to use to

obtain the statistical results. Refer to the presentation in Chapter 2 to assist you in developing the statistical treatment of the data.

References

1. Annual Book of ASTM Standards, Part 31, Water, 1980. Philadelphia: American Society for Testing Materials, 1980, pp 541–549.
2. Harris DC. Quantitative Chemical Analysis. 3rd ed. San Francisco: WH Freeman, 1991, p 542.
3. Pietrzyk D, C Frank. Analytical Chemistry. 2nd ed. New York: Academic Press, 1979, p 658.

INTRODUCTION TO THE VISIBLE SPECTROPHOTOMETER

Background and Summary of Method

The simple visible spectrophotometer has been an important instrument in trace environmental analysis for many years. Colorless environmental contaminants must be chemically converted to a species which appears colored to the eye. The colored species will then exhibit regions of the visible electromagnetic spectrum where it will absorb photons. This absorption can be related to the Beer–Lambert or Beer-Bouguer Law of Spectrophotometry according to:

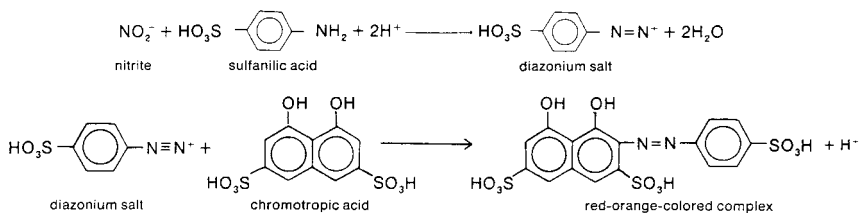
$$A = \epsilon bc$$

where A is the absorbance measured in absorbance units (AUs) and, in turn, is related to the logarithm of the ratio of incident to transmitted intensity, ϵ is the molar absorptivity, a unique property of a chemical substance, viewed as a constant in the equation, b is the path length in either millimeters or centimeters, assumed to be a constant (see cuvette matching below), and c is the concentration in either molarity or weight/unit volume, if known; this forms the basis for calibration of UV–vis and atomic absorption spectrophotometers.

Recall that the color of a solution is the complement of the color of light that it absorbs. For example, the red cobalt chloride solution you will be using to conduct the cuvette matching (see below) actually absorbs the complement to red, which is green at a wavelength of 510 nm. The term “spectrophotometer” succinctly and completely describes the instrument. “Spectro” refers to the visible spectrum and the ability of the instrument to select a wavelength or, more accurately, a range of wavelengths. “Photo” refers to light and “meter” implies a measurement process. Some instruments lack the capability of selecting a wavelength and should be called “photometers.” A good question to ask when using a spectrophotometer

is: "What is the precision in nanometers when I set the wavelength?" The answer should be as follows: "It depends on the effective bandpass of the monochromator." The effective bandpass is a measure of the band of wavelengths allowed to pass through the spectrophotometer for a given width of the exit slit of the monochromator. The bandpass is given by the product of the slit width and the reciprocal linear dispersion. The theory of light dispersion on gratings and prisms and subsequent effects on monochromator resolution has been well developed (1, 2).*

The major limitation on the use of visible spectrophotometric or colorimetric methods is the fact that the analyte must absorb within the visible domain of the electromagnetic spectrum. Many analytes of environmental interest are colorless. Many can be chemically converted to a colored product by direct chemical change of the analyte, formation of a metal chelate, or formation of an ion pair. An example of a chemical conversion of the analyte of interest is the colorimetric determination of nitrite-nitrogen via reaction of the nitrite ion with sulfanilic acid in acidic media to form the diazonium salt with subsequent reaction with chromotropic acid to form a highly colored azo dye.



An example of metal chelation formation can be found if one refers to *Standard Methods for the Examination of Water and Wastewater* for the recommended methods for determining the toxic metal cadmium. An atomic absorption method is listed along with a dithizone method. Cd^{2+} combines with dithizone (HDz) in aqueous media to form the neutral, colored molecule $\text{Cd}(\text{Dz})_2$. This neutral molecule can be subsequently extracted into a non-polar solvent thus providing an increase in the method

*A number of good presentations can be found on the basic principles of spectrophotometry, refer to the references in Chapter 4. A particularly good presentation is found in Chapter 19 of Harris D. Quantitative Chemical Analysis. 3rd ed. San Francisco: Freeman, 1991. Also consider Skoog D, J Leary. Principles of Instrumental Analysis. 4th and 5th eds. Philadelphia: Saunders. Chapters 2 and 3 of Robinson J. Undergraduate Instrumental Analysis. 5th ed. (New York: Marcel Dekker, Inc., 1995), provides a good introduction. A more comprehensive treatment is found in Ingle J, Crouch S. Spectrochemical Analysis. Englewood Cliffs, NJ: Prentice-Hall, 1988.

detection limit (MDL). The extent to which a given metal ion can be chelated with HDz and subsequently extracted is defined as the distribution ratio, D_M , for a given metal ion and depends on the formation constant of the complex, the overall extraction constant, the initial concentration of HDz in the organic phase, the fraction of the total metal concentration present as the divalent cation, and, most importantly, the pH of the aqueous solution. For the equilibrium



the distribution ratio is expressed mathematically as (refer back to Eq. 3.57)

$$D_M = \frac{\beta_n K_{ex} [HDZ]_o^n}{[H^+]^n} \alpha_M$$

An example of a chelated ion-association pair which when formed is subsequently extracted into a nonpolar solvent is that of the iron(II)-*o*-phenanthroline cation which efficiently partitions into chloroform provided a large counterion such as perchlorate is present in the chloroform.

Experimental

A possible major source of error in spectrophotometric measurement occurs when the path length differs from one sample to the next [i.e., the value for b (see above) is different]. Hence, two solutions having identical concentrations of a colored analyte (i.e., both a and c are equal) could exhibit different absorbances due to differences in path length b . To minimize this source of systematic error, a cuvette matching exercise is introduced. Cuvette tubes are matched by placing a solution of intermediate absorbance in each and comparing absorbance readings. One tube is chosen arbitrarily as a reference and others are selected that have the same reading to within 1%. Tubes should always be matched in a separate operation before any spectrophotometric experiments are begun.

Glassware Needed per Student or Group

Seven to ten 13×100 -nm glass test tubes.

Chemical Reagents Needed per Student or Group

Ten milliliters of a 2% cobalt chloride solution: dissolve 2 g of $CoCl_2 \cdot 6 H_2O$ in approximately 100 mL of 0.3M HCl. This solution is recommended because it is stable, has a broad absorption band at about

the center of the visible region, and transmits about 50% in a 1-cm cell. Each group should prepare this solution and share among members.

Miscellaneous Item Needed per Student or Group

One piece of chalk which nicely fits into a 13×100 -mm test tube.

Spectrophotometer

Any stand-alone UV-vis absorption spectrophotometer can be used to perform this exercise. In college laboratories, the Spectronic 20[®] is commonly found. In recent years, the series of GENESYS[®] instruments from Spectronic Instruments Inc. is finding increasing use in analytical labs.

Procedure (3)

Obtain a supply of 13×100 -mm test tubes that are clean, dry, and free of scratches. Half-fill each tube with the 2% CoCl_2 solution. Set the wavelength to 510 nm on the spectrophotometer; then, zero the readout. Choose one tube as a reference, place a vertical index mark near the top of the tube, and insert the tube into the sample compartment. Adjust the light control so that the meter reads 90% transmittance (T). Insert each of the other tubes and record their transmittances. If the % T is within 1% of the reference tube, place an index mark so that the tube can be inserted in the same position every time. If it is not within 1%, rotate the tube to see whether it can be brought into range. In future measurements, insert each tube in the same position relative to its index mark. Choose a set of seven tubes that have less than 1% variation in reading. Retain these tubes for subsequent photometric work and return the remainder. To compensate for variations between instruments, use the same instrument for both tube matching and experimental work.

To show that as the wavelength cam is manually rotated, different wavelengths are passed across the exit slit by the monochromator, place a piece of chalk into a test tube and insert into the sample compartment while leaving the cover open. Starting at 400 nm, scan the visible range up to 700 nm and observe the exit-slit image by looking straight down into the cell compartment as you rotate the cam.

For the Report

Because this is an introductory exercise, there is no report.

References

1. Ewing G. Instrumental Methods of Chemical Analysis. 5th ed. New York: McGraw-Hill, 1985, pp 23–31.

2. Vandecasteele C, C Block. Modern Methods for Trace Element Determination. New York: John Wiley & Sons, 1993, pp 86–89.
3. Harris W, B Kratochvil. An Introduction to Chemical Analysis. Philadelphia: Saunders, 1981, pp 410–412.

**USING THE PROGRAM “LSQUARES” to ESTABLISH THE LEAST SQUARES
REGRESSION LINE FOR VISIBLE SPECTROPHOTOMETRIC DETERMINATIONS**

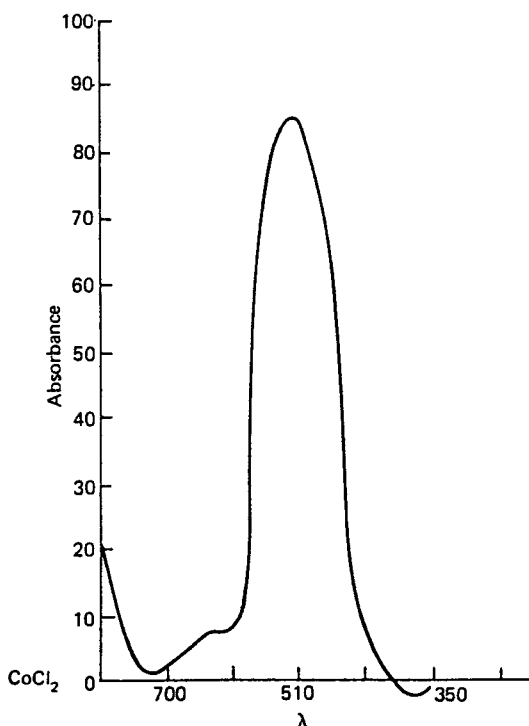
Typical calibration data obtained from a visible spectrophotometer for aqueous solutions which contain dissolved cobalt chloride, CoCl_2 :

Concentration (mg/L or ppm) $\text{CoCl}_2(\text{aq})$	Absorbance (absorbance units, A.U.)
500	0.100
600	0.235
700	0.415
800	0.610
900	0.795
1000	1.010

Use “LSQUARES” to find the best straight line fit through the experimental x, y data points.

At the wavelength used in the measurement, a portion of a solution whose concentration is unknown is added to a cuvette, placed in the spectrophotometer, and the absorbance measured. Assuming that the unknown solute responsible for the color [in this case the aquated cobalt(II) ion] is the same chemical species in which the previously prepared calibration plot was made, find the concentration in mg/L or ppm from the interpolated least squares regression plot if the absorbance measured is 0.755 A.U.

The following graph is the visible absorption spectrum for Co^{2+} . What wavelength should be used to conduct this quantitative analysis?



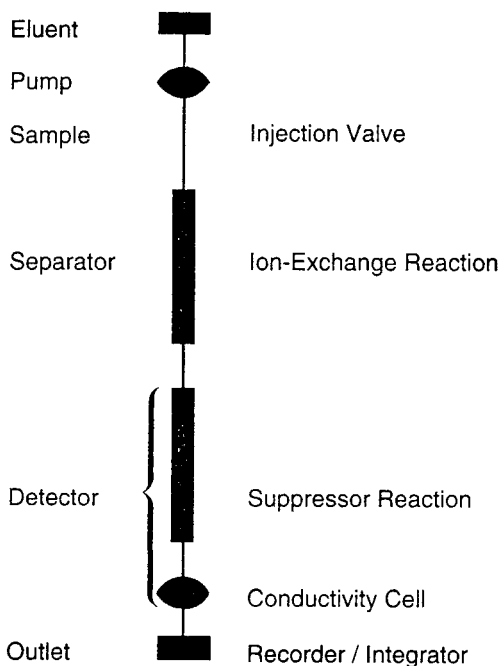
**DETERMINATION OF INORGANIC ANIONS USING ION CHROMATOGRAPHY (IC):
ANION-EXCHANGE IC WITH SUPPRESSED CONDUCTIVITY DETECTION**

Background

An aqueous sample obtained from the environment that may be expected to contain dissolved inorganic salts can be injected into a properly installed and optimized ion chromatograph. The concentration of the most common inorganic anions and corresponding cations can be quantitatively determined. The instrumentation available in our laboratory is that manufactured by the Dionex Corporation in the early to mid-1980s.

Ion chromatography (IC) is a low- to moderate-pressure liquid chromatographic (LC) technique and should be clearly distinguished from that of high-pressure LC (HPLC). IC as a determinative technique has been developed to separate both cations and anions. The instrument available to the student utilizes anion-exchange IC with suppressed conductivity detection. This technique can separate the *common inorganic anions*: **fluoride**,

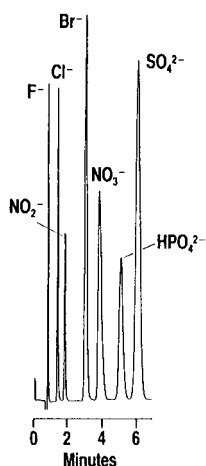
chloride, bromide, nitrite, nitrate, phosphate, and sulfate under the IC conditions used here. A second set of related anions of environmental interest has emerged in recent years and these are collectively called *inorganic disinfection by-products* and include **bromate, bromide, chlorite, and chlorate**. EPA Method 300.1 has been recently revised and this method addresses both sets of analytes. Prior to the development of the micromembrane suppressor, a suppressor column was used to reduce the background conductivity of the mobile-phase eluent. The following is a schematic drawing of the classical ion chromatograph:



For anion-exchange IC, the eluent must be a moderate to strong base (e.g., carbonate or hydroxide). Carbonate or hydroxide ions displace the analyte ion through the anion-exchange resin in the separator column. The separator must contain a low-capacity anion-exchange resin so that the analyte ions can make it through the column in a reasonable length of time after injection. Because the conductivity detector responds to all ions, a strong signal due to the eluent would be observed, thus “swamping out” the contribution due to the analyte ions. These eluent ions can be chemically removed while the analyte ions elute from the suppressor in a low-conductivity background. This is done in the suppressor and the conductivity of the

eluent is said to be “chemically suppressed.” The suppressor and conductivity cell comprise the IC detector as shown above. The suppressor must contain a cation-exchange substrate whereby H^+ from the regenerant migrates across the membrane (which is itself a cation exchanger) and neutralizes the carbonate or hydroxide to form neutral carbonic acid or water. The micromembrane suppressor requires a continuous supply of regenerate solution that consists of 0.025*N* sulfuric acid. The regenerant used in our laboratory utilizes mid-1980s technology and consists of a reservoir that contains the dilute H_2SO_4 . This solution is made to flow into and out of the micromembrane suppressor by means of positive air pressure. More contemporary designs self-generate the H_2SO_4 electrochemically.

A typical ion chromatogram for separation and detection of a reference standard that contains all seven *common* anions follows:



Anion Standard

Conditions

Separator: AS4A
Suppressor: AMMS
Eluant: 2mM Na_2CO_3 ,
0.75mM $NaHCO_3$
Flow Rate: 2.5 mL/min

Concentrations

Anion	PPM
F^-	1
Cl^-	2
NO_2^-	3
Br^-	10
NO_3^-	10
HPO_4^{2-}	10
SO_4^{2-}	15

How Do I Operate a Typical Ion Chromatograph?

To operate the ion chromatograph, read the operator's manual. Alternatively, your instructor may have a written procedure. For purposes of illustration, we list below a procedure used in our lab. A Model 2000 (Dionex) instrument interfaced to a PC via a 900 (PE-Nelson) interface is available:

1. Turn the compressed air valve on and adjust the main pressure gauge to 80 psi. This provides a head pressure to the eluent reservoir.

2. Adjust the small pressure gauge located adjacent to the ion chromatograph to approximately 10 psi. This provides a head pressure to the regenerant reservoir.
3. Turn on the power to the chromatography module/SP via a switch located in the rear of the module. This will start the single-piston reciprocating pump on the Model 2000.
4. Turn the conductivity detector cell display to “on” and monitor the output. This is located on the detector module. A reading between 10 and 20 μS with a tolerance of $< 1 \mu\text{S}$ represents a stable baseline. This implies that the ion chromatograph is sufficiently equilibrated across the micromembrane suppressor. At times, the regenerate flow rate may need to be increased or decreased by adjusting the head pressure. If regenerate is not flowing through the micromembrane suppressor, the conductivity value will skyrocket!
5. At the PC workstation, retrieve the appropriate method from Turbochrom. The document Anion.mth is available if a more specific method has not been created.
6. Either create a sequence or use under “setup” the method and proceed to download the method. This enables the instrument and the PC to communicate.
7. Once the workstation PC reaches a ready status, press inject to remove the lit LED on the Dionex Automation Interface. Fill the 50- μL injection loop which is located at the sample port on the chromatography/SP with a filtered, aqueous sample. Press the “inject” and immediately proceed to the 900 interface box (PE-Nelson) and press “start.”
8. After the chromatographic run is complete, repeat Step 7 by first depressing the “inject” to remove the LED.

Is There a Need for Sample Prep?

Aqueous samples that are free of suspended-matter and contain dissolved inorganic salts are the only type of sample matrix that is suitable for direct injection into the IC. Wastewater samples that contain suspended solids must be filtered prior to injection into the IC. Wastewater samples that have a dissolved organic content [i.e., an appreciable total organic carbon (TOC)] level, should be passed through a previously conditioned reversed-phase solid-phase extraction (RP-SPE) cartridge to attempt to remove the dissolved organic matter prior to injection into the IC. Keep in mind that these RP-SPE cartridges have a finite capacity. If this capacity is exceeded, contaminants will merely pass through. If samples come from a bioreactor,

proteins and other high-molecular-weight solutes must also be removed prior to injection into the IC.

After implementing the operations procedure, the eluent should be pumping through the separator column and the micromembrane suppressor while the regenerant should be flowing in the opposite direction to the eluent flow through the suppressor under building supplied compressed air. The ion chromatograph is interfaced to a PC workstation that uses Turbochrom (PE-Nelson) chromatography data acquisition and control software via a 900 interface box.

Also included in this experiment are specific procedures to prepare the bicarbonate/carbonate eluent, the preparation of the mixed anion reference standards, and weights of various salts to be used to prepare stock reference standard solution for all analytes of interest.

How Do I Prepare a Reference Stock Standard for Each Anion?

The weight of each salt that can be used to prepare a 1000 ppm as the anion without any reference to a cation is listed in the following table:

Salt	g to dissolve to prepare 1 L of a 1000 ppm as X
NaF	2.210
NaCl	1.646
KBr	1.488
NaNO ₂	1.500
NaNO ₃	1.371
K ₂ SO ₄	1.814
KBrO ₃	1.315
KClO ₃	1.467
NaClO ₂	1.341

How Do I Prepare the Bicarbonate/Carbonate Eluent "From Scratch?"

Dissolve 0.571 g of NaHCO₃ and 0.763 g of Na₂CO₃ in approximately 250 mL of distilled, deionized water (DDI). Transfer this solution to a 2000-mL graduated cylinder. Adjust to the mark with DDI and transfer this solution to the IC reservoir. Add 2000 mL more of DDI to the IC reservoir for a total of 4 L. Label the IC reservoir as "1.7 mM HCO₃/1.8 mM CO₃" eluent. This eluent is used with either a AS4A (Dionex) or AN 300 Sarasep (Metachem) anion-exchange IC separator column.

How Do I Prepare a Mixed Anion Stock Standard for IC?

The following table outlines one approach to prepare a mixed stock reference standard:

mL of 1000-ppm stock	Anion	Conc. (ppm)
3.0	Chloride	3.0
1.0	Nitrite	1.0
10.0	Bromide	10
2.0	Nitrate	2.0
20.0	Phosphate	20.0
10.0	Sulfate	10.0

A 1000-mL volumetric flask is used. Pipette the indicated aliquot into the flask that is approximately one-half filled with DDI.

How Do I Prepare a Four-Level Set of Calibration Standards for IC?

Using the stock solution, proceed using the following table as a guide to prepare a set of working calibration standards:

mL stock	V (mL)	Cl	NO ₂	Br	NO ₃	HPO ₄	SO ₄
5.0	25	0.6	0.2	2.0	0.4	4.0	2.0
10.0	25	1.2	0.4	4.0	0.8	8.0	4.0
20.0	25	2.4	0.8	8.0	1.6	16.0	8.0
Neat	—	3.0	1.0	10.0	2.0	20.0	10.0

What Do the Data Look Like?

Four ion chromatograms are included in this experiment that were run on the Model 2000 instrument. Figure 5.5 shows a separation of six of the seven common anions only 5 min after the instrument was turned on. Note the gradual rise in the baseline during development of the chromatogram. This same reference standard of six anions was injected long after the baseline had stabilized and is shown in Fig. 5.6. A stable baseline is essential for reproducible peak areas and hence good precision and accuracy for TEQA. The removal of bromide ion from a three-anion mix when passed through a conditioned resin coated with silver nitrate (SPE cartridges manufactured by Alltech) is evident when ion chromatograms shown in Figs. 5.7 and 5.8 are compared. It is evident that bromide is partially removed, whereas nitrite and nitrate (anions that do not form insoluble precipitates with Ag⁺) are not.

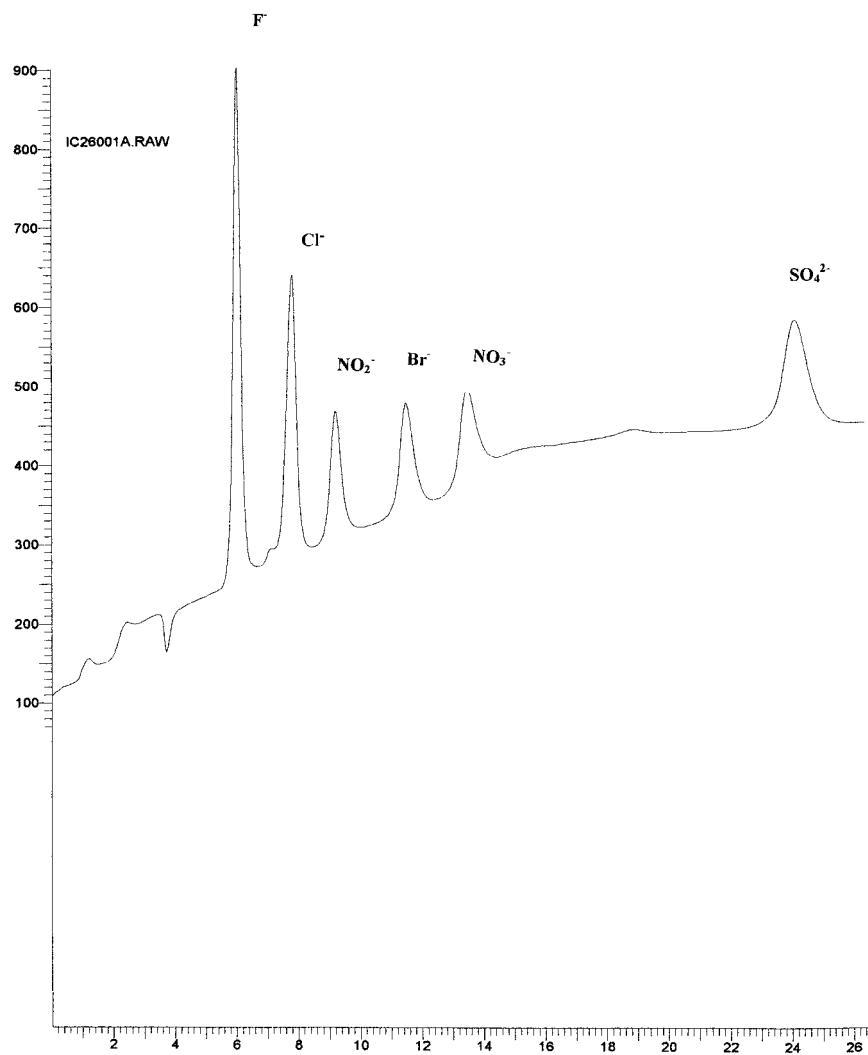


FIGURE 5.5 Direct injection after only 5 min since instrument was turned on.

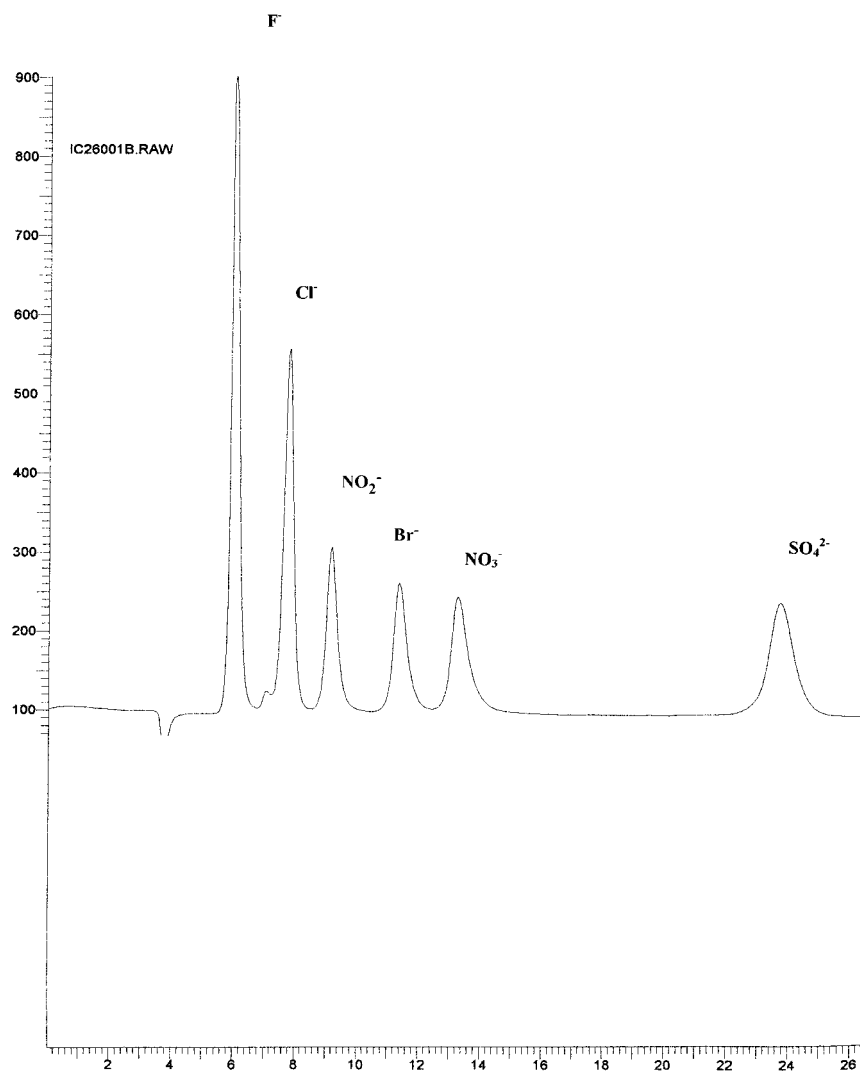


FIGURE 5.6 Direct injection of reference standard containing common inorganic ions.

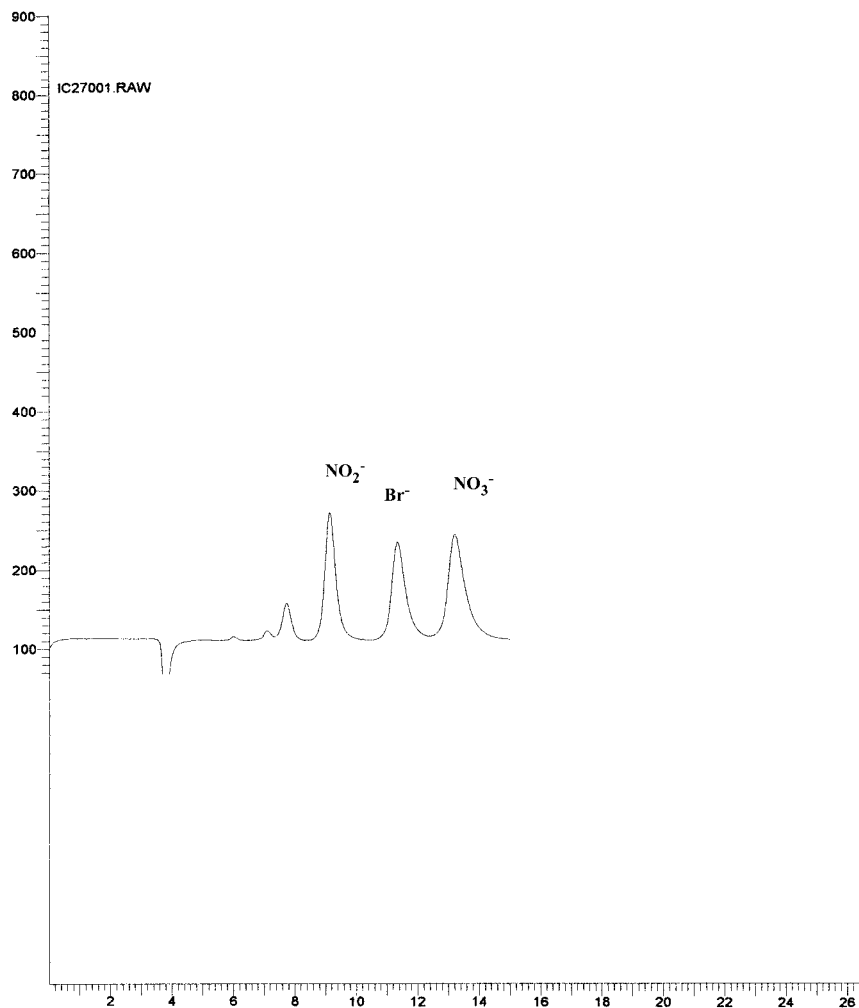


FIGURE 5.7 Direct injection of aqueous solution containing sodium salts of NO_2^- , Br^- , and NO_3^- .

What Tasks Do I Perform with IC?

Your instructor will provide you with directions as to what to do. You may be asked to use the ion chromatograph to provide a quantitative analysis of one or more of the *common* anions found in a groundwater sample. This exercise may be incorporated as part of your study of snow and contaminated soil. If a quantitative analysis is required, you must calibrate the IC

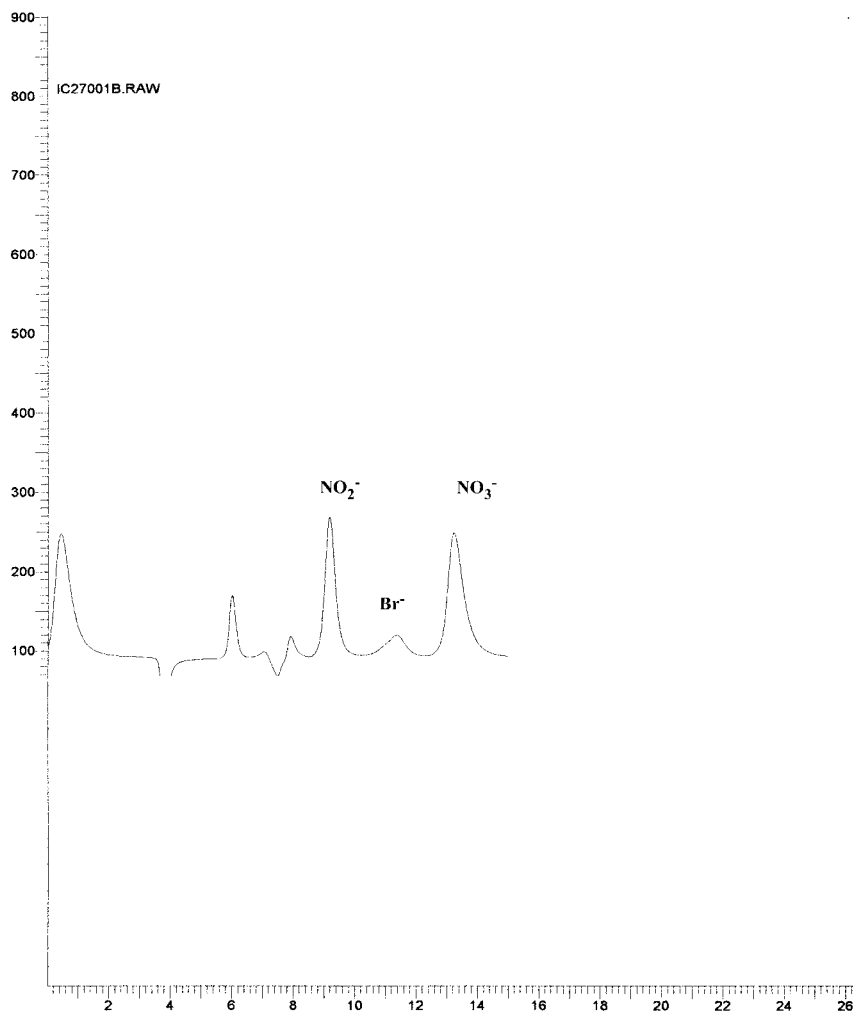


FIGURE 5.8 Injection of aqueous solution after passing through IC Ag^+ .

using the above table as a guide. In our laboratory, the IC is interfaced via a 900 (PE-Nelson) to a PC that runs Turbochrom (PE-Nelson). Once a calibration curve is established using Turbochrom, sample analysis can be accomplished using the appropriate report format within the software.

Where Can I Read More About IC?

The principles underlying IC can be found in any number of introductory texts discussed in the references on IC found in Chapter 4. Suppliers of ion chromatographs, IC columns, and related accessories such as Dionex Corporation and Alltech Associates are good sources of technical notes and applications.

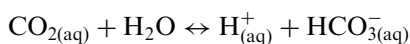
AN INTRODUCTION TO pH MEASUREMENT: ESTIMATING THE DEGREE OF PURITY OF SNOW; MEASURING SOIL pH; ION CHROMATOGRAPHY

Background and Summary of Method

The laboratory will focus on the operational aspects of pH measurement. It is appropriate that we start this course with pH because this parameter is so fundamental to the physical–chemical phenomenon that occurs in aqueous solutions. The pH of a solution which contains a weak acid determines the degree of ionization of that weak acid. Of environmental importance is an understanding of the acidic properties of carbon dioxide. The extent to which gaseous CO_2 dissolves in water and equilibrates is governed by the Henry law constant for CO_2 . We are all familiar with the “carbonation of beverages.” The equilibrium is



When CO_2 is dissolved in water, a small fraction of the dissolved gas exists as carbonic acid, H_2CO_3 . The acid–base character of CO_2 may be described by the following reactions and equilibrium constants:



$$K_{a1} = \frac{[\text{H}^+][\text{HCO}_3^-]}{[\text{CO}_2]} = 4.45 \times 10^{-7} \quad (5.1)$$

$$\text{p}K_{a1} = 6.35$$



$$\text{p}K_{a2} = 10.33$$

For example, the molar concentration of CO_2 in water saturated with this gas at a pressure of 1 atm at 25°C is $3.27 \times 10^{-2} M$. The pH, using Eq. (5.1),

can be calculated and is 3.92. Environmental water samples generally have less than the saturated molarity value and yield a pH of approximately 5.9. Thus, the difference between ultrapure water with a theoretical pH of 7 and water exposed to the atmosphere with a measurable pH of 5.9 is attributed to dissolved CO_2 and its formation of carbonic acid, which, in turn, dissociates to hydronium, bicarbonate, and carbonate ions. The degree to which carbon dioxide remains in its molecular form or exists as either bicarbonate or carbonate depends upon the pH. The fraction, α , of each of these species of the total is mathematically defined as

$$\alpha_{\text{CO}_2} = \frac{[\text{CO}_2]}{[\text{CO}_2] + [\text{HCO}_3^-] + [\text{CO}_3^{2-}]} \quad (5.3)$$

$$\alpha_{\text{HCO}_3^-} = \frac{[\text{HCO}_3^-]}{[\text{CO}_2] + [\text{HCO}_3^-] + [\text{CO}_3^{2-}]} \quad (5.4)$$

$$\alpha_{\text{CO}_3^{2-}} = \frac{[\text{CO}_3^{2-}]}{[\text{CO}_2] + [\text{HCO}_3^-] + [\text{CO}_3^{2-}]} \quad (5.5)$$

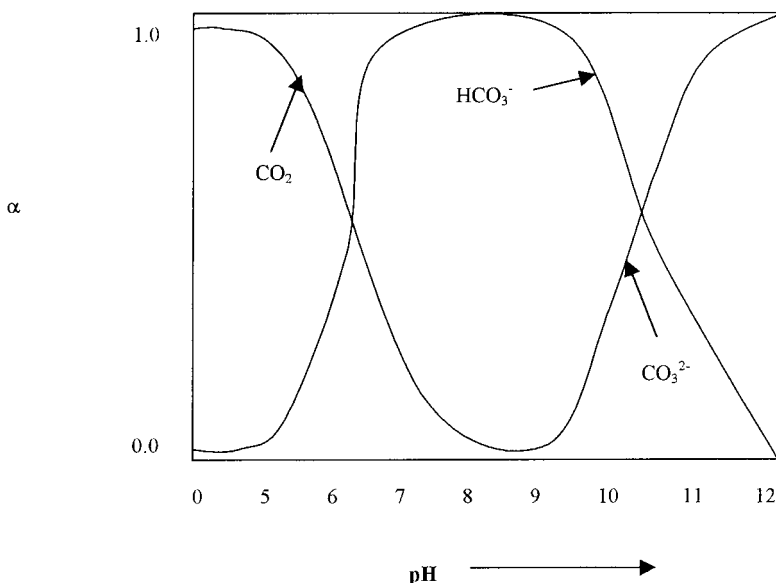
Equations (5.3)–(5.5) can be reworked in terms of acid dissociation constants and hydronium ion concentrations to yield

$$\alpha_{\text{CO}_2} = \frac{[\text{H}^+]^2}{[\text{H}^+]^2 + K_{a1}[\text{H}^+] + K_{a1}K_{a2}} \quad (5.6)$$

$$\alpha_{\text{HCO}_3^-} = \frac{K_{a1}[\text{H}^+]}{[\text{H}^+]^2 + K_{a1}[\text{H}^+] + K_{a1}K_{a2}} \quad (5.7)$$

$$\alpha_{\text{CO}_3^{2-}} = \frac{K_{a1}K_{a2}}{[\text{H}^+]^2 + K_{a1}[\text{H}^+] + K_{a1}K_{a2}} \quad (5.8)$$

Equations (5.6)–(5.8) can be used to construct what are called *distribution of species diagrams*, which are plots of the fraction of each species as a function of solution pH. For the $\text{CO}_2/\text{HCO}_3^-/\text{CO}_3^{2-}$ system, the distribution of species diagram is as follows:



The amount of acidic solutes or alkaline solutes present in a given volume of a groundwater, surface-water, or wastewater sample is determined by conventional acid–base titration. Refer to *Standard Methods for the Examination of Water and Wastewater* for specific procedures. Titrations are performed in these methods to a specified pH. Be sure to distinguish among the concepts of acidity, alkalinity, and pH when considering the nature of environmental samples. The pH will need to be measured and adjusted prior to conducting the ion-pair liquid–liquid extraction for determining methylene blue active surfactants.

The pH was originally measured by judicious choice of acid–base indicator dyes and has in recent years given way to potentiometric methods due to the inherent limitations of color. A dye serves no useful purpose when the wastewater sample is light brown. Advances in both the instrument as well as the glass electrode have taken the measurement of pH very far since the early days when Arnold Beckman in 1935 was first asked to measure the pH of a lemon.

Experimental

Glassware Needed per Student

- 1 250- or 500-mL beaker to sample snow
- 1 50-mL beaker
- 1 Stirring rod

Chemical Reagents Needed per Student

Each pH measurement station should consist of the following:

1. Three buffer solutions of pH 4, 7, and 10, respectively
2. One squeeze bottle containing distilled, deionized water (DDI)
3. One waste beaker
4. Training guide for operating the Orion SA 720 pH/ISE Direct Readout Meter

Procedure

Use the guide located at each of four pH measurement stations and familiarize yourself with the operation of the pH meter. Each student should calibrate the meter using the pH 4 and pH 7 buffer solutions that are available by implementing the autocalibration. An ATC probe will not be available; therefore, samples and standards should be at the same temperature. Use a stirring rod to continuously stir the solution in the 50-mL beaker while a pH measurement is being taken.

Obtain at least three samples of snow. One sample should be obtained as close to a walkway or roadway and appear visibly “dirty.” One sample should be obtained as far away from pollutant sources and appear visibly “clean.” Use a relatively large beaker to collect snow and allow the snow to melt. **Record pH values in your notebook.** Draw conclusions about your observations and write these in your laboratory notebook.

Obtain one or more soil samples from a hazardous waste site. Alternatively, your instructor may have a series of fortified soils available in the laboratory. An illustration of how a series of laboratory fortified acidic, neutral, and alkaline soils can be prepared and given to students as sample unknowns is shown in Table 5.3. Implement EPA Method 9045C while reviewing its details. Assume that the unknown soil is *noncalcareous*. Notice the use of flowcharts in helping to understand the procedural aspects of the method. Repeat the pH measurement for the four other samples and **record your results** in your laboratory notebook. The chemical nature of the contaminated soil samples will be revealed to you after you have completed your pH measurements. Rationalize the observed pH value for each soil based on a knowledge of the chemical used to contaminate the soil. Write your comments into your lab notebook.

As an additional feature to this experiment, the environmental engineering staff will have a Dionex 2000i Ion Chromatograph in operation. This instrument will separate and detect inorganic anions such as chloride, nitrate, and sulfate. Are these chemical species present in the various snow samples? In what manner do inorganic anions contribute to snow acidity?

TABLE 5.3

Sample No.	Soil type	Chemical added
1	Tappan B	Salicylic acid
2	Tappan B	Sodium carbonate
3	Tappan B	None
4	Hudson River Sediment, naturally contaminated with PCBs	None
5	Unknown sandy soil	Citric acid
6	Unknown sandy soil	Potassium hydrogen phthalate
7	Unknown sandy soil	None
8	Unknown sandy soil	<i>p</i> -Nitrophenol
9	Unknown sandy soil	Tetrabutylammonium hydroxide

You have generated hazardous waste from your soil pH measurements. Dispose of properly in accordance with the Office of Radiation, Chemical and Biological Safety (ORCBS) guidelines.

Suggested Readings Related to this Experiment

Many of the more lucid presentations on pH are found in general chemistry texts, whereas instrumental concepts of pH measurement are found in analytical chemistry texts. The most definitive text at an advanced level is Bates, R. *Determination of pH* (New York: John Wiley & Sons, 1964) and is located in most chemistry libraries.

A useful guide to the measurement of soil pH in soil is found in *Test Methods for Evaluating Solid Waste: Physical/Chemical Methods, SW-846* (Washington, DC: Environmental Protection Agency, 1995, Volume 1, Section C, Method 9045C, Revision 3).

Appendix A: Glossary

Trace environmental quantitative analysis (TEQA) has developed a “terminology of its own” and this appendix consists of a glossary of terms used in not only trace analysis but also in the regulatory realm. The following sources were used to develop this glossary.

- Environmental Testing and Analysis News, 22–8 (February 1995) (overall regulatory and generic trace analysis terms)
- Erickson, M. Analytical Chemistry of PCBs. Lewis Publishers, 1997, Appendix H (terms related to the determination of PCBs)
- Anderson, R. Practical Statistics for Analytical Chemists. New York: Van Nostrand Reinhold, 1987, pp 51–54 (terms related to the statistical definitions)

Accuracy A measure of how close a measured value is to a known true value. Accuracy is assessed by means of reference samples and percent recoveries of spiked samples.

Aliquot A measured portion of a sample taken for analysis.

Alternative hypothesis Usually comes about from the logic of statistical testing. One example of an alternative hypothesis (refer to the definition of a **null hypothesis**) is to state that the precision of population A is *not*

equal to that of population B. If the two variances are not equal, the nonequality may be stated mathematically in three ways: (a) $H_A: \sigma_A^2 \neq \sigma_B^2$, (b) $H_A: \sigma_A^2 < \sigma_B^2$, and (c) $H_A: \sigma_A^2 > \sigma_B^2$. The first inequality is “two sided,” meaning that the inequality can be approached in either direction, whereas the second and third inequalities are “one sided.” If the first inequality is of interest, a “two-tailed” *F*-test (for variances) or a “two-tailed” *t*-test (for means) is most appropriate. For the second or third inequalities, a “one-tailed” *F*- or *t*-test is most appropriate.

Analyte The chemical element or compound an analyst seeks to determine; the chemical element of interest.

Analytical batch The basic unit of analytical quality control, defined as samples that are analyzed together with the same method sequence and the same lots of reagents and with the manipulations common to each sample within the same time period or in continuous sequential time periods. Samples in each batch should be of similar composition (e.g., groundwater, sludge, ash).

Analytical sample Any solution or media introduced into an instrument on which an analysis is performed, excluding instrument calibration, initial calibration verification, initial calibration blank, continuing calibration blank. The following are all analytical samples: undiluted and diluted samples, predigestion spike samples, duplicate samples, serial dilution samples, analytical spike samples, postdigestion spike samples, interference check samples, laboratory control samples, preparation or method blank, and linear range analysis samples.

Analysis The ascertainment of the identity and/or the concentration of the constituents or components of a sample. Analysis is often used correctly in place of determination. Only samples can be analyzed; constituents or components as determined. Examples of correct usage are analysis of fish for PCBs and determination of PCBs in fish.

Appendix VIII This list in 40 CFR Part 261 contains 355 compounds and classes of compounds shown to be toxic, carcinogenic, mutagenic, or teratogenic in reputable scientific studies.

Appendix IX This list in 40 CFR Part 264 replaced Appendix VIII with regard to the groundwater monitoring regulations found in 40 CFR 264 and 40 CFR 270.

Area units A term used in gas chromatography which indicates the peak area of a compound exiting a chromatographic column. The size or area of the peak is proportional to the amount of analyte in the sample.

Aroclor Trade name (Monsanto, St. Louis, MO) for a series of commercial PCB and polychlorinated terphenyl mixtures marked in the United States.

Askarel A general term for a group of nonflammable, synthetic, chlorinated, aromatic hydrocarbons used as electrical insulating media.

Atomic absorption (AA) A technique for analyzing metals using an element-specific lamp that emits a characteristic light spectrum. A sample is heated in a flame or graphite furnace and the light beam is passed through it. When the sample absorbs light, an energy loss is detected and is translated into a concentration of metal in the sample. This technique detects one metal at a time.

Audit A systematic check to determine the quality of some function or activity. Two basic types are performance audits and system audits. Performance audits involve a quantitative comparison of the laboratory's results to those of a proficiency sample containing known concentrations of analytes. A system audit is a qualitative evaluation that normally consists of an on-site review of a lab's quality assurance system and physical facilities.

Background correction A technique usually employed relative to metals analysis which compensates for variable background contribution to the instrument signal in the determination of trace elements.

Batch See **Analytical batch**.

Ballshmitter number Serial numbering system for PCBs from 1 to 209, starting with 2-monochlorobiphenyl and ending with decachlorobiphenyl; also termed "IUPAC No."

Bioassay The use of living organisms to measure the effect of a substance, factor, or condition by comparing before and after data. The term is often used to mean cancer bioassays.

Biochemical oxygen demand (BOD) A measure of the amount of oxygen consumed in the biochemical processes that break down organic matter in water. A larger BOD value indicates a greater degree of organic pollution. BOD₅, a related term, is the amount of dissolved oxygen consumed in 5 days.

Blank An artificial sample designed to monitor the introduction of artifacts into the measurement process. For aqueous samples, reagent water is used as a blank matrix. A universal matrix does not exist for solid samples; therefore, no matrix is routinely used. There are several types of blanks, which monitor a variety of processes:

- A *laboratory blank* is taken through sample preparation and analysis only. It is a test for contamination in sample preparation and analyses.

- A *holding blank* is stored and analyzed with samples at the laboratory. It is a test for contamination in sample storage as well as sample preparation and analysis.
- A *trip blank* is shipped to and from the field with the sample containers. It is not opened in the field and, therefore, provides a test for contamination from sample preservation, site conditions, and transport as well as sample storage, preparation, and analysis. It is most commonly used for volatile organics.
- A *field blank* is opened in the field and tests for contamination from the atmosphere as well as those activities listed under *trip blank*.

BNA Base neutral, and acid extractable compounds. The terms *base*, *neutral*, and *acid* refer to the pH condition of the sample undergoing extract more efficiently from water under acidic conditions. Such compounds are often referred to as “acid extractables.”

4-Bromofluorobenzene (BFB) A compound utilized in EPA gas chromatography/mass spectrometry (GC–MS) volatile methods to establish mass spectral instrument performance. It is also used as a surrogate for volatile organic analysis.

Calibration The systematic determination of the relationship of the response of the measurement system to the concentration of the analyte of interest. Instrument calibration performed before any samples are analyzed is called the initial calibration. Subsequent checks on the instrument calibration performed through analysis are called continuing calibration.

California list Created by the state of California; this list is used to determine those wastes that are restricted from land disposal. The list was incorporated by RCRA.

CAS Registry Number Unique number assigned by the Chemical Abstracts Service to each chemical compound.

CERCLA The Comprehensive Environmental Response, Compensation and Liability Act, also known as “Superfund.” Enacted December 11, 1980, and amended thereafter, CERCLA provides for identification and cleanup of hazardous materials released over the land and into the air, waterways, and groundwater. It covers areas affected by newly released materials and older leaking or abandoned dump sites. CERCLA established the Superfund, a trust fund, to help pay for cleanup of hazardous materials sites. The EPA has the authority to collect cleanup costs from those who release the waste material. Cleanup funds come from fines and penalties, from taxes on chemical/petrochemical feedstocks, and the U.S. Department of the Treasury. A separate fund collects taxes on active disposal sites to finance monitoring after they close.

Chain-of-custody Procedures and associated documents designed to trace the custody of a sample from the point of origin to final disposition, with the intent of legally demonstrating that custody remained intact and that tampering or substitutions were precluded.

Chemical oxygen demand (COD) A measure of the oxygen required to oxidize all compounds in water, both organic and inorganic.

Chlorinated hydrocarbons Organic compounds containing one or more chlorine atoms. These include a class of persistent, broad-spectrum insecticides that linger in the environment and accumulate in the food chain. Among them are DDT, aldrin, dieldrin, heptachlor, chlordane, lindane, endrin, mirex, hexachloride, and toxaphene. Another example is trichloroethene (TCE), an industrial solvent.

Clean Water Act (CWA) Regulates the discharge of nontoxic and toxic pollutants into surface waters. The CWA (Public Law 92-500) became effective November 18, 1972 and has been amended significantly since then. Its ultimate goal is to eliminate all discharges into surface waters usable for fishing, swimming, and other beneficial uses. The EPA and the Army Corps of Engineers have jurisdiction. The EPA sets guidelines and state agencies issue permits (e.g., National Pollutant Discharge Elimination System permits), specifying the types of control equipment and allowable discharges for each facility.

Code of Federal Regulations (CFR) A collection of the federal regulations established by law and published by the Government Printing Office.

Colorimetric Analyses based on the measurement of the color that develops during the test-specific reaction. The intensity of color development is usually measured at a specified wavelength on a spectrophotometer.

Confirmation In GC, an unknown compound in a sample is identified on the basis of its retention time on a specific chromatographic column. Because several compounds may exhibit that exact same retention time on a given column, a secondary confirmation on a different column or detector is often recommended for additional confidence in the compound identification. This additional confirmation is often referred to as “dual-column” or “dual-detector” confirmation.

Congener One of 209 PCBs or other group of compounds, not necessarily the same homolog.

Continuous liquid-liquid extraction An extraction technique that involves boiling the extraction solvent in a flask and condensing the solvent above the aqueous sample. The condensed solvent drips through the sample and extracts the compounds of interest from the aqueous phase.

Contract Laboratory Program (CLP) A program coordinated through the EPA to provide a wide range of analytical services by commercial laboratories in support of investigation, remediation, and enforcement actions at Superfund sites. Laboratories participating in this program are under contract to the EPA and must follow very specific analytical protocols during analyses and data delivery, as specified in the statement of work associated with the contract.

Coplanar Those PCB congeners lacking or containing only one chlorine in the *ortho* positions. Chlorine substituents are larger than hydrogens and thus constrain rotation about the carbon–carbon bond bridging the two rings in the biphenyl. PCBs with zero or one *ortho*-chlorine can form a coplanar conformation more readily than those congeners with two, three, or four chlorines in the *ortho* positions. The mono-*ortho*-chloro congeners may or may not be included in the term “coplanar,” depending on the authors of the study.

Data quality objectives (DQO) During the planning phase of a project requiring laboratory support, the data user must establish the quality of data required from the investigation. Such statements of data quality are known as DQOs. Qualitative and quantitative statements about the data required to support specific decisions or regulatory actions, DQOs must take into account sampling considerations as well as analytical protocols.

Data validation A systematic effort to review data for identification of errors and thereby deleting or flagging suspect values to assure the validity of the data for the user. This process may be done by manual or computer methods.

Decafluorotriphenylphosphine (DFTPP) An organic compound utilized in several EPA GC–MS methods to establish proper mass spectral instrument performance for semivolatile analyses.

Determination The ascertainment of the quantity or concentration of a specific substance in a sample.

Digestion log An official record of the sample preparation procedures used in processing a sample prior to instrumental analysis. This is most often associated with digestion of samples utilizing various acids prior to analysis for metals.

Dissolved metals Metallic elements determined on a water sample that has been passed through a 0.45- μm filter.

Dissolved oxygen (DO) The oxygen freely available in water. DO is vital to fish and other aquatic life and for the prevention of odors. Traditionally, the level of DO has been accepted as the single most important indicator of a water body’s ability to support desirable aquatic life. Secondary and

advanced waste treatment are generally designed to protect DO in waste-receiving waters.

Dissolved solids Disintegrated organic and inorganic material contained in water. Excessive amounts make water unfit to drink or use in industrial processes.

Dry weight The weight of a sample based on percent solids; also, the weight of a sample after drying in an oven at a specified temperature.

Effluent Treated or untreated wastewater that flows out of a treatment plant, sewer, or industrial outfall. Generally refers to wastes that are discharged into surface waters and are regulated under the Clean Water Act. Effluent limitations are restrictions on quantities, rates, and concentrations of wastewater discharges that are established by a state or the EPA.

Electron-impact mass spectrometry Low-resolution mass spectrometry operated in the electron-impact ionization mode.

External standards A method of quantifying chromatographic data in which standards of known concentrations are analyzed prior to unknown samples. The chromatographic peak area (or height) of a sample component is compared to a calibration curve of a peak area constructed from the standard data for that component. This comparison allows the concentration of the component in the sample to be determined.

Fecal coliform bacteria Bacteria found in mammals' intestinal tracts. Their presence in water or sludge is an indicator of pollution and possible contamination by pathogens.

Field screening An investigative technique utilizing analytical chemistry at or near a work site to rapidly determine the presence or absence of environmental contaminants and the approximate concentration of specific target compounds.

Flame ionization detector (FID) A GC detector in which the column effluent gas is mixed with hydrogen and burned in air or oxygen. The ions and electrons produced in the flame generate an electric current proportional to the amount of material in the detector. The FID responds to nearly all organic compounds, but it does not respond to air and water, which makes it exceptionally suited to environmental samples.

Flash point The lowest temperature at which a flammable liquid gives off sufficient vapor to form an ignitable mixture with air near its surface or within a vessel. Combustion does not continue.

Gas chromatograph (GC) A technique for detecting organic compounds by using their physical and chemical properties to separate a mixture. The

compounds are identified and quantified with various types of detectors as they exit the chromatograph. Selection of detectors is dependent on the particular compounds of interest.

Gas chromatography–mass spectrometry (GC–MS) A technique in which sample analytes are bombarded with electrons as they exit a GC column and are fragmented into characteristic ion patterns. The mass spectrometer is the detector. It can determine which fragments are present and, therefore, the identity of the compounds.

Graphite furnace A technique used for the analysis of metals. An AA spectrophotometer heats the sample within a graphite tube using an electrical current. It is also commonly called a flameless furnace and generally provides greater sensitivity for certain metals than flame or inductively coupled argon plasma techniques.

Gravimetric Analyses based on the direct or indirect weighing of the analyte in question. This technique usually requires the use of an analytical balance with a sensitivity of 0.1 mg or better.

Hall electrolytic conductivity detector An element-selective GC detector primarily intended for trace analysis of organic compounds containing chlorine, nitrogen, or sulfur. In operation, this detector pyrolyzes the column effluent gas into soluble electrolytes that are dissolved in a stream of deionized liquid. The observed change in electrical conductivity, proportional to the amount of material present, is measured.

Hazardous Ranking System (HRS) The principal screening tool used by EPA to evaluate risks to public health and environment associated with abandoned or uncontrolled hazardous waste sites. The HRS calculates a score based on the potential for hazardous substances spreading from the site through the air, surface water, or groundwater and on other factors such as nearby population. This score is the primary factor in deciding if the site should be on the National Priorities List and, if so, what ranking it should have there.

Hazardous substance Any material that poses a threat to human health and/or the environment. Typical hazardous substances are toxic, corrosive, ignitable, explosive, or chemically reactive.

Hazardous waste Waste regulated under RCRA that can pose a substantial or potential hazard to human health or the environment when improperly managed. Such wastes possess at least one of our characteristics (ignitability, corrosivity, reactivity, or toxicity) or appear on special EPA hazardous waste lists. The term is not interchangeable with hazardous substance or material.

Headspace Any area in a container not completely filled by the sample in which gases can collect.

Heavy metals Metallic elements with high atomic weights (i.e., mercury, chromium, cadmium, arsenic, and lead). They can damage the health of plants and animals at low concentrations and tend to accumulate in the food chain.

Holding time The storage time allowed between sample collection and sample analysis when the designated preservation and storage techniques are employed.

Homolog One of the 10 degree of chlorination of PCBs ($C_{12}H_9Cl$ through $C_{12}Cl_{10}$) or other group of compounds varying by systematic addition of a substituent.

Hydrocarbons (HC) Chemical compounds that consist entirely of carbon and hydrogen.

ICP Inductively coupled argon plasma (also referred to as ICAP). An instrument used for metals analysis. Because the temperature of the plasma is considerably higher (10,000 K) than the temperature of a flame AA spectrophotometer, it is especially useful for refractory metals. Some instruments are also capable of performing simultaneous multi-element analysis.

Ignitable Capable of burning or causing a fire.

Inorganic chemicals Chemical substances of mineral origin, unlike organic chemicals whose structure relies on carbon atoms.

Instrument detection limit (IDL) A term utilized in the EPA Inorganic Contract Laboratory Program. The IDL is three times the standard deviation obtained for the analysis of a standard solution (each analyte in reagent water) at a concentration of three to five times that of the IDL on three nonconsecutive days with seven consecutive measurements per day.

Instrument tuning A technique used in GC-MS procedures to verify that the instrument is properly calibrated to produce reliable mass spectral information.

Internal standards Compounds added to every standard, blank, matrix spike, matike spike duplicate, sample (for volatile organics), and sample extract (for semivolatiles) at a known concentration, prior to analysis. Internal standards are used as the basis for quantification of the target compound.

Ionization Utilized in mass spectrometry to fragment analyze molecules into smaller segments. These smaller mass segments are then separated

and plotted to form a “mass spectrum,” which is used to identify the parent molecule. Electron impact is one example of ionization used in mass spectrometry. In more technical terms, ionization is the process by which neutral atoms or groups of atoms become electrically charged, either positively or negatively, by the loss or gain of electrons.

Isomer Chemical compounds with the same molecular weight and atomic composition but differing molecular structure (e.g., *n*-pentane and 2-methylbutane).

Land ban The 1984 Resource Conservation and Recovery Act amendments mandated that by May 1990 all untreated hazardous waste must be banned from land disposal. The treatment standard and concentration levels were implemented in “thirds” beginning in November 1986.

Leachate A liquid that results from water collecting contaminants as it trickles through wastes, agricultural pesticides, or fertilizers. Leaching may occur in farming areas, feedlots, and landfills and may result in hazardous substances entering surface water, groundwater, or soil.

Library search A technique in which an unknown mass spectrum of a compound is compared to the mass spectra of compounds contained in a computer library in an effort to identify the compound. Compounds identified in this manner are referred to as tentatively identified compounds (TICs).

Liquid chromatography (LC) A chromatographic separation technique in which the substance to be analyzed is dissolved in a solvent and, using the same or a different solvent, is eluted through a solid adsorbent exhibiting differential adsorption for the components of the substance. The technique is analogous to GC except that heat is not necessarily required for the separations to take place. This technique is quite useful in analyzing compounds.

Listed waste Any waste listed as hazardous under the Resource Conservation and Recovery Act but which has not been subjected to the Toxic Characteristics Listing Process because the dangers it presents are considered self-evident.

Log-in The receipt and initial management of an environmental sample. It generally includes identifying who sent the sample, maintaining chain-of-custody, checking report and invoice information, recording analyses requested, including methodology and special instructions, and assigning a discreet in-lab identification, usually a number or bar code.

Mass spectrum A plot of ion mass-charge ratio versus intensity. A fragmentation pattern results from the impact on a given molecule of a beam of electrons. The impact produces a family of particles whose mass dis-

tribution is characteristic of the parent molecule. Qualitative information is provided by a mass spectrum.

Material Safety Data Sheet (MSDS) A compilation of information required under the OSHA Communication Standard on the identity of hazardous chemicals and their associated health and physical hazards, exposure limits, and precautions.

Matrix The physical characteristics or state of a sample (e.g., water, soil, sludge).

Matrix interference The influence of the sample matrix or sample components on the ability to qualitatively identify and quantitatively measure compounds in environmental samples.

Matrix modifiers Chemicals added to samples for metals analysis which are used to lessen the effects of chemical interferences, viscosity, and surface tension.

Matrix spike Aliquot of a sample fortified (spiked) with known quantities of specific compounds and subjected to the entire analytical procedure in order to indicate the appropriateness of the method for the matrix by measuring recovery of the spike.

Matrix spike duplicate A second aliquot of the same matrix as the matrix spike that is spiked to determine the precision of the method.

Maximum contaminant level (MCL) The maximum permissible level of a contaminant in water delivered to any user of a public water system. MCLs are enforceable standards.

Method detection limit (MDL) The minimum concentration of a compound that can be measured and reported with 99% confidence that the value is above zero.

Narrative In an analytical report, a descriptive documentation of any problems encountered in processing the samples, along with corrective action taken and problem resolution.

National Pollutant Discharge Elimination System (NPDES) A provision of the Clean Water Act that prohibits discharge of pollutants into waters within the United States unless a special permit is issued by the EPA, a state (where delegated), or a tribal government on an Indian reservation.

Null hypothesis Usually comes about from the logic of statistical testing. One example of a null hypothesis is to state that the precision of population A is equal to that of population B. It is given by a symbol as H_0 and stated mathematically as $\sigma_A^2 = \sigma_B^2$. To answer the question, "are my two variances really different" is rephrased statistically as "are the two variances equally precise?"

Nutrient Any substance assimilated by living things that promotes growth. The term is generally applied to nitrogen and phosphorus in wastewater, but it is also applied to other essential and trace elements.

Organic Generally, any compound that contains carbon bonded to a hydrogen or halogen atom.

Oxidation The process in chemistry whereby electrons are removed from a molecule.

Part per million One part in 10^6 . For a low concentration of analyte in an aqueous sample, a weight/volume (w/v) basis is most commonly used and $1 \text{ ppm} = 1 \text{ mg/L}$. For nonaqueous liquids and solids, a weight/weight (w/w) basis is most commonly used and $1 \text{ ppm} = 1 \text{ mg/kg}$.

PCBs Polychlorinated biphenyls, a group of toxic persistent chemicals used in transformers and capacitors for insulating purposes and in gas-pipeline systems as a lubricant. Sale of PCBs for new uses was banned by law in 1979.

Percent recovery A measure of accuracy that is calculated as the measured value relative to the true value, expressed as a percent.

Performance audit A quantitative evaluation of a measurement system that involves the analysis of standard reference samples or materials which are certified as to their chemical composition or physical characteristics.

Petrochemicals Chemicals derived from the refining of hydrocarbons (oil and natural gas). Plastics are created through processing of petrochemicals, making them valuable as a fuel in waste-to-energy incineration facilities.

Petroleum hydrocarbon fingerprinting A method that identifies sources of oil and allows spills to be traced back to their source.

pH A numerical designation of relative acidity and alkalinity. A pH of 7.0 indicates precise neutrality. Progressively higher values indicate increasing alkalinity and lower values increasing acidity.

Pollutant Generally, any substance introduced into the environment that adversely affects the usefulness of a resource.

Potentially responsible party (PRP) Any individual or company—including owners, operators, transporters or generators—potentially responsible for, or contributing to, the contamination problems at a Superfund site. Whenever possible, EPA requires PRPs, through administrative and legal actions, to clean up sites they have contaminated.

Practical quantitation limit (PQL) The lowest level that can be reliably achieved within specified limits of precision and accuracy during routine laboratory operating conditions.

Precision A measure of the ability to reproduce analytical results. It is generally determined through the analysis of duplicate samples.

Preservative A chemical or reagent added to a sample to prevent or slow decomposition or degradation of a target analyte or a physical process. Physical and chemical preservation may be used in tandem to prevent sample deterioration.

Primary Drinking Water Regulation Applies to public water systems and specifies a contaminant level that, in the judgment of the EPA Administrator, will have no adverse effect on human health.

Procedure The written directions necessary to use a method or series of methods and techniques.

Protocol A sampling or analysis procedure which is highly specific and from which few or no deviations are allowed.

Purge and trap A technique used in the analysis of volatile organics where analytes are purged from a sample by means of an inert gas and trapped on a sorbent column. The sorbent is then flash-heated and the analytes are transferred onto a GC column for separation and identification.

Purgeable organic An organic compound that is generally less than 2% soluble in water and has a boiling point at or below 200°C; a volatile organic. An organic compound is generally considered to be purgeable if it can be removed from water using the purging process.

Qualitative Having to do with establishing the presence or identity of a compound.

Quality assurance (QA) All those planned and systematic actions necessary to provide adequate confidence in laboratory results.

Quality assurance program plan A written assembly of management policies, objectives, principles, and general procedure that outlines how the laboratory intends to generate data of known and accepted quality.

Quality control (QC) Those quality assurance actions that provide a means to control and measure the characteristics of measurement equipment and processes in order to meet established requirements.

Quantitative Having to do with measuring the amount or concentration of a compound in a sample.

Quantitation limit The minimum concentration of a compound that can be reliably quantified; very dependent on the sample matrix. *See* **Practical quantitation limit**.

Quantitative analysis An analysis to measure or determine the amount of a target analyte with a set degree of precision and accuracy.

Reactivity The tendency of a chemical to explode under normal management conditions, to react violently when mixed with water, or to generate toxic gases.

Reagent water Water in which an interference is not observed at or above the minimum quantitation limit of the parameters of interest.

Reconstructed ion chromatogram (RIC) A mass spectral graphical representation of the separation achieved by a GC indicating total ion current versus retention time.

Relative response factor (RRF) A measure of the relative mass spectral response of an analyte compared to its internal standard. RRFs are determined by analysis of standards and are used in the calculation of concentrations of analytes in samples.

Relative retention time (RRT) Retention time of a compound on a chromatographic system, relative to an internal standard; unitless number.

Resolution The degree of separation between peaks eluting from a chromatographic column. Sufficient resolution between peaks is required for proper quantitation of unknown analytes.

Retention index Systematic, unitless measure of a compound's chromatographic retention as compared to an homologous series of standards, usually the *n*-alkanes.

Resource Conservation and Recovery Act (RCRA) A federal law that established a regulatory system to track hazardous substances from the time of generation to disposal. The law requires safe and secure procedures to be used in treating, transporting, storing, and disposing of hazardous substances. RCRA is designed to prevent new and uncontrolled hazardous waste sites.

Retention time A term used in GC and in HPLC describing the time elapsed from sample injection until the specific compound elutes or exits the chromatographic column at the detector. Each compound has a characteristic retention time on a specific column; therefore, this information is used to qualitatively identify the compounds in the sample.

Secondary Drinking Water Regulations Unenforceable regulations that apply to public water systems and specify contamination levels that, in the judgement of the EPA, are required to protect the public welfare. These regulations apply to any contaminants that may adversely affect the odor or appearance of such water and, consequently, may cause people served by the system to discontinue its use.

Skinner list Created by John Skinner of the EPA Office of Solid Waste; a list of those compounds most often found in petroleum refining wastes.

Solid waste Nonliquid, nonsoluble materials, ranging from municipal garbage to industrial wastes, that contain complex, and sometimes hazardous, substances. Solid wastes include sewage, sludge, agricultural refuse, demolition wastes, mining residues, and even liquids and gases in containers.

Solvent A substance, usually liquid, capable of dissolving or dispersing one or more other substances.

Standard curve A curve that plots concentrations of known analyze standards versus the instrument response to the analyze. Calibration standards are prepared by diluting the stock analyte solution in graduated amounts that cover the expected range of the samples being analyzed. The calibration standards must be prepared by using the same type of acid or solvent at the same concentration as for the samples following sample preparation. This is applicable to organic and inorganic chemical analyses.

Standard operating procedure (SOP) A written quality assurance document which describes the way an organization typically conducts a routine activity. It may address instrumental operation, instrument maintenance, application of a laboratory technique, data review, or management oversight.

Statistically significant When the difference between a predicted and an observed value is so large that it is improbable that it could be attributed to chance.

Statistical testing Proposing a *null hypothesis* and an *alternative hypothesis*. The logic of statistical testing as discussed by Anderson is as follows:

- Formulate a null hypothesis and an alternative hypothesis
- Define the population (or universe) and state any assumptions
- Plan the experiment
- Define the basis for decision making
- Run the experiment as planned
- Evaluate the data
- State the conclusion

Our decision to accept or reject a null hypothesis may be a correct one; that is, we may accept a true null hypothesis or reject a false null hypothesis or it may be a wrong one. Rejecting a true null hypothesis is called committing a *Type I error*. Accepting as true a false hypothesis is called committing a *Type II error*. These possibilities can be summarized in a *truth table*.

Superfund The Response Trust Fund, established by CERCLA, as a mechanism for the federal government to take emergency or remedial action to clean up both abandoned and existing disposal sites when there is a release, or potential threat of a release, of a hazardous substance presenting imminent and substantial danger to public health and welfare; *see* CERCLA.

Surrogate An organic compound similar to the analyte of interest in chemical composition, extraction, and chromatography, but not normally found in environmental samples. Primarily used in chromatography techniques, the surrogate standard is spiked into quality control blanks, calibration and check standards, samples (including duplicates and QC reference samples), and spiked samples before analysis. A percent recovery is calculated for each surrogate.

Suspended solids Small pollutant particles that float on the surface of, or are suspended in, sewage or other liquids. They resist removal by conventional means.

Target Compound List (TCL) A list of organic compounds that are determined during Superfund site remediations. Created by the EPA for use in the Contract Laboratory Program, this list was formerly referred to as the Hazardous Substance List (HSL).

Target compounds specific compounds that are to be quantified in a sample, based on a standard list of potential compounds.

Technique Scientific principle or specific operation.

Tentatively identified compounds (TICs) Compounds detected in samples that are not target compounds, internal standards, system monitoring compounds, or surrogates. TICs usually consist of up to 30 peaks that are greater than 10% of the peak areas, or heights, of the nearest internal standard. They are subjected to mass spectral library searches for tentative identification. A client may specify the number of unknown peaks in its samples it wishes the laboratory to tentatively identify.

Total metals Metallic elements that have been digested prior to analysis.

Trihalomethane (THM) One of a family of organic compounds with derivatives of methane. THMs are generally by-products of the chlorination of drinking water that contains organic material.

Truth table (related to the logic of statistical testing) The probability p of making a Type I error is α and, thus, the probability of making a correct decision when the null hypothesis is true is $1 - \alpha$. Similarly, the probability of making a Type II error when the null hypothesis is false is β and the

probability of making a correct decision is $1 - \beta$. This logic is summarized in the following *truth table*:

	What the analyst thinks	What the scientific facts suggest
Decision	H_0 is true	H_0 is false
H_0 is true	Correct $p = 1 - \alpha$	Type II error $p = \beta$
H_0 is false	Type I error $p = \alpha$	Correct $p = 1 - \beta$

Trust fund A fund set up under the Comprehensive Environmental Response, Compensation and Liability Act (or equivalent state Superfund law) to help pay for cleanup of hazardous waste sites and for legal action to force those responsible for them to clean them up.

Underground storage tank A chemical tank located entirely or partially underground.

Vadose zone Unsaturated soil (i.e., above the water table).

VOA Volatile organic analysis, also refers to volatile organic acids.

VOA bottle A vial used to contain samples for volatile organic analysis.

Volatile compounds Compounds amenable to analysis by purge and trap. Synonymous with purgeable compounds.

Volatile organic compound (VOC) Any organic compound that participates in atmospheric photochemical reactions, except for those designated by the EPA Administrator as having negligible photochemical reactivity. A subset of VOCs that include one or more chlorine atoms covalently bonded to carbon and are abbreviated, at least in this book, as CIVOCs. Examples of CIVOCs are vinyl chloride, trichloroethene (TCE), and perchloroethylene or tetrachloroethylene (PCE).

Wet chemistry Procedures that involve distillations, colorimetric determinations, and titrimetric measurements. Examples of analytes that are routinely quantitatively determined by wet chemical methods include cyanides, methylene blue active surfactants, biological and chemical oxygen demands, abbreviated BOD, and COD.

Appendix B: QA/QC Illustrated

Trace environmental quantitative analysis (TEQA) requires that a specific QC document be written and available. This appendix further elaborates on QC as first introduced in Chapter 2 and first written by the author in support of the National Institutes of Environmental Health Sciences (NIEHS) Basic Superfund Research Center Analytical Core.

1. WHAT DOES A SPECIFIC QC PLAN LOOK LIKE?

Many of the concepts introduced in this chapter will now be applied in the example that follows. The standard operating procedure (SOP) and QC document that follows provides guidelines for the quantitative determination of trace concentrations of either organochlorine or polychlorinated biphenyl targeted analytes that were routinely performed in the past in the author's laboratory. The sample matrix was composed of rat plasma and the sample preparation method used consisted of ultrasonic probe sonication (PS) using acetonitrile followed by solid-phase extraction in the reversed-phase mode (RP-SPE) using either an octyl (C_8) chemically bonded silica or an octadecyl (C_{18}) chemically bonded silica. The analytes were retained on the RP silica, the RP silica was dried, and then eluted using pesticide-residue grade iso-octane. After adjustment to a precise 1-mL eluent

volume, 1 μL of eluent was injected into a previously calibrated gas chromatograph.

2. HOW IS A QC/SOP PLAN ORGANIZED?

A good QC/SOP document tells the client exactly how the analysis will be conducted and how the analytical data produced by execution of the procedures will be interpreted. The document illustrated here is presented in the following sequence:

1. Title
2. Summary
3. Percent recovery study
4. Calibration, verification, and quantitative analysis
5. Establishment of the IDL
6. Establishment of the MDL
7. QC definitions
8. QC specifications
9. Reporting requirements

2.1 Title: Trace Organochlorine Pesticide (OCs)/ Polychlorinated Biphenyls (PCBs) Analysis via PS Coupled to RP-SPE Using Capillary Gas Chromatography with Electron-Capture Detection (C-GC-ECD):SOP and QC Protocols

2.2 Summary of Method

Samples of biological origin such as plasma, serum, organ parts, and so forth that arrive in the laboratory are immediately frozen or refrigerated as recommended by the client. Prior to beginning the analysis, the samples are thawed and brought to ambient temperature. The primary technique used in our laboratory to isolate and recover the target OC or PCB analytes from the biological matrix is ultrasonic probe sonication combined with reversed-phase solid-phase extraction (PS-RP-SPE). A 10-cartridge vacuum manifold is used. This enables up to 10 SPE extractions to be performed simultaneously. In addition to the number of samples that are to be analyzed, one sample must be selected and two identical aliquots of this sample are needed. The first additional sample is needed to spike (matrix spike) and the second additional sample identical to the first is needed to spike again (matrix spike duplicate). If these additional sample matrices are not available for conducting recovery studies, matrix spikes will not be included.

2.3 Percent Recovery Study

A percent recovery study will be conducted. A surrogate reference standard consists of a methanolic 2-ppm solution that contains tetrachloro-*m*-xylene (TCMX) and decachlorobiphenyl (DCBP). Surrogates are added to all samples, whereas matrix spikes are added to only selected samples. A matrix spike reference standard consists of a methanolic 24-ppm solution that contains PCB congeners or specific Aroclors. A control sample is prepared by taking the same aliquot of surrogate and of matrix spike and placing this in the 1-mL volumetric flask and adjusting the final volume to 1.0 mL. A series of SPE cartridges used to conduct this study are used. In conjunction with a 10-cartridge vacuum manifold (e.g., Vacmaster, International Sorbent Technology, Ltd), a method development study is listed according to the following table:

SPE No.	μL 2-ppm surrogate	μL 24-ppm matrix spike	Matrix	Description
1	100	0	ACN	Method Blank
2	100	100	ACN	Blank Spike-1
3	100	100	ACN	Blank Spike-2
4	100	100	ACN	Blank Spike-3
5	100	100	Sample	Matrix Spike
6	100	100	Sample	Matrix Spike Duplicate
7	100	0	Sample	Sample-1
8	100	0	Sample	Sample-2
9	100	0	Sample	Sample-3
10	100	0	Sample	Sample-4
—	100	100	Iso-octane	Control

A 1-mL eluent is obtained from PS–RP–SPE that is obtained from PS–RP–SPE that is quantitatively transferred to a clean, dry, 2-mL GC autosampler glass vial with a crimped top cap. Percent recoveries are routinely found to be between 75% and 100% for most OCs and PCBs from a biological matrix used to conduct toxicological studies. The appropriate GC method is retrieved from the chromatography software that controls and acquires data from the GC (Turbochrom, PE-Nelson). The order in which the vials are injected into the G–GC–ECD is called a sequence. A sequence file is created using the Turbochrom chromatography processing software (PE-Nelson). Several QC protocols are used to consider the order in which sample eluents from PS–RP–SPE are injected into a C–GC–ECD. Blanks are run before and after standards. Usually, injection of a standard that has a high concentration of analyte is followed by injection of a blank to mini-

mize carryover. A representative sample sequence is given in the following table:

Injection vial No.	Description of GC vial
1	Blank (pure iso-octane)
2	Control (100% recovery)
3	Blank (pure iso-octane)
4	SPE method blank
5	SPE blank spike-1
6	SPE matrix spike
7	Blank (pure iso-octane)
8	SPE sample unspiked-1

Injection vials 1–8 represent the first cycle in a series of several cycles in which a vial containing pure iso-octane is first injected to estimate whether there is any carryover from previous injections. Then, a control vial is injected that contains the analyte of interest and represents a 100% recovery. Pure iso-octane is then injected a second time in this cycle to prevent carryover from the high concentration of analyte in the control. A method blank is then injected that demonstrates that the method itself is free from laboratory and analyst contamination. This is followed by an injection of the blank spike eluent in order to assess the % recovery of analyte from a relatively clean matrix. This is followed by an injection of the matrix spike eluent in order to assess the % recovery of analyte from the sample matrix itself. A matrix effect can then be evaluated from these two spiked samples. To prevent carryover again, pure iso-octane is injected. The sample itself, unspiked, is then injected to provide sample analysis. A second cycle is then undertaken in a similar matter as just introduced for the first cycle. If there are sufficient samples, a third, fourth, and even a fifth cycle are also introduced.

2.3.1 Calculation of Analyte Percent Recovery

The integrated peak areas for all targeted analytes over replicate injections of the control and spikes are then averaged and used to calculate a mean percent recovery according to

$$\%R_i = \frac{\sum_i^N A_i^s / N}{\sum_i^n A_i^c / n} \times 100$$

where A_i^s represents the integrated peak area for the i th component in the spiked sample or spiked blank, A_i^c represents the integrated peak area for the i th component in the control sample, N represents the number of repli-

cate spiked samples or blanks, and n represents the number of replicate control injections into the GC.

2.4 Calibration, Verification, and Quantitative Analysis

Preparation of the Stock Solution

Weigh 0.0500 g (does not have to be exact, record in notebook) of each OC, PCB congener, or Aroclor. Place the solid or liquid into a 10-mL volumetric flask half-filled with methanol (MeOH, use highest purity available). Adjust to a final volume with the bottom of the minusus horizontally aligned with the calibration mark. This yields a 5000-ppm stock solution. Transfer to a clean, dry, glass vial with a Teflon septum and screw cap. Label the vial clearly as “5000 ppm ... (MeOH), date”. Store at refrigerated temperatures.

Preparation of the Primary Dilution

Place 100 μL of stock solution into a clean dry 10 mL volumetric flask which is half-filled with MeOH. Adjust to a final volume with MeOH and transfer to a storage vial and label “50 ppm ...”

Preparation of the Secondary Dilution

Place 100 μL of primary dilution into a clean, dry 10-mL volumetric flask which is half-filled with MeOH. Adjust to final volume with MeOH and transfer to a storage vial and label “500 ppb ...”

Preparation of the Tertiary Dilution

Place 100 μL of secondary dilution into a clean, dry 10-mL volumetric flask which is half-filled with MeOH. Adjust to final volume with MeOH and transfer to a storage vial and label “5 ppb ...”

Preparation of the Working Calibration Standards

Use the following table to prepare a series of working calibration standards using MeOH as the dilution solvent. Transfer the standards to a 2-mL GC vial, cap, and either place on autosampler or store for future use.

Standard	μL 5 ppb	V (total)	Conc. (ppb)
1	5	1.0	0.025
2	10	1.0	0.050
3	50	1.0	0.250
4	100	1.0	0.500
5	250	1.0	1.250
6	500	1.0	2.500

A multipoint calibration curve using the ES mode is established for each OC and PCB congener or total Aroclor using either the secondary or tertiary diluted reference standards (depending on the range of concentration desired). The working calibration standards are prepared originally from either a chemically pure form of the OC or PCB or from a commercially available and certified solution. A least square regression analysis is performed using the available software or via in-house computer programs and the quality of the least squares fit is evaluated in terms of its correlation coefficient and standard deviations in both the slope and intercept. An initial calibration verification (ICV) standard is prepared and injected in triplicate to evaluate the precision and accuracy of the least squares regression. The confidence interval for the ICV is then established. Peak areas for the separated and detected OCs, PCB congeners, or total Aroclors are converted to their corresponding concentration levels expressed either in ppb or ppm of each analyte or as total Aroclor. A confidence interval about the interpolated concentration level is also obtained. For example, the 3,4,3',4'-tetrachlorobiphenyl congener present in a sample of rat plasma is reported as being 76 ± 5 ppb at the 95% significance level.

2.5 Establishment of the Instrument Detection Limit

The IDL is defined as the minimum concentration or weight of analyte that can be detected at a known confidence level. This limit depends on the ratio of the magnitude of the analytical signal to the size of the statistical fluctuations in the blank signal.

The IDL is then defined as that concentration which corresponds to the ratio of the difference between the minimal distinguishable analytical signal and the mean signal in a blank to the slope of the least squares regression line (calibration sensitivity). Because this minimum distinguishable signal can either be defined as being equal to the sum of the mean signal from a blank S_{blank} and either 3 or 10 times the standard deviation in the blank signal, $\text{std dev}_{\text{blank}}$, two definitions must be used: the minimum detectable limit C_{IDL} and the limit of quantitation C_{LOQ} . Expressed mathematically,

$$S_{\text{IDL}} = \bar{S}_{\text{blank}} + 3s_{\text{blank}}$$

$$C_{\text{IDL}} = \frac{S_{\text{IDL}} - S_{\text{blank}}}{\text{Slope}}$$

$$S_{\text{LOQ}} = \bar{S}_{\text{blank}} + 10s_{\text{blank}}$$

$$C_{\text{LOQ}} = \frac{S_{\text{LOQ}} - S_{\text{blank}}}{\text{Slope}}$$

Hence, C_{IDL} and C_{LOQ} yield two levels that must be considered when defining detection limits. Because the sample preparation technique involves the isolation and recovery of analytes into an extract from the original sample, a concentration factor as well as a consideration of the efficiency of the extraction process as defined by the percent recovery is involved. Thus, the method detection limit (MDL) will be lower than the IDL.

2.6 Establishment of the MDL

The MDL can be found only after the IDL, the concentration factor (F), and the percent recovery R (expressed as its decimal equivalent) are determined for the method. F is the ratio of sample volume V_S to extract volume V_{eluent} . The volume of rat plasma, V_s , is usually 1 or 2 mL, whereas V_{eluent} is 1.0 mL. Expressed mathematically,

$$C_{MDL} = \frac{C_{IDL}F}{R}$$

where

$$F = \frac{V_S}{V_{eluent}}$$

2.7 Definitions of QC Reference Standards

Surrogate spiking standard Consists of tetrachloro-*m*-xylene (TCMX) and decachlorobiphenyl (DCBP) dissolved in MeOH. These two analytes are highly chlorinated and are structurally very similar to OCs and PCB targeted analytes. TCMX elutes prior to and DCBP after the OCs and PCBs, thus eliminating any coelution interferences. A fixed volume (aliquot) of this reference solution is added to every sample, matrix spike, blank, blank spike, and control in the protocol.

Matrix spike standard Consists of a representative set of the targeted analytes (i.e., OCs and/or PCBs dissolved in MeOH). A precise aliquot of this solution is added to a sample so that the effect of the sample matrix on the percent recovery can be evaluated.

Control standard Consists of the same representative set of targeted analytes used in the matrix spike and the same precise aliquot of this solution is added to an aliquot of the elution solvent used in PS-RP-SPE and is used in the calculation of percent recoveries.

Stock reference standard The highest concentration of target analyte obtained by either dissolving a chemically pure form of the analyte in an appropriate solvent or obtaining it commercially as a certified reference solution.

Primary dilution standard Results from dilution of the stock reference standard.

Secondary dilution standard Results from dilution of the primary dilution standard.

Tertiary dilution standard Results from dilution of the secondary dilution standard.

Instrument calibration verification standard (ICV) Prepared in a similar manner as for the working calibration standards, however at a different concentration than any of the working standards. Used to evaluate the precision and accuracy for an interpolation of the least squares regression of the calibration curve.

Calibration or working standards A series of diluted standards prepared from dilutions made from the tertiary dilution standard. These standards are injected directly into the C–GC–ECD and used to calibrate the instrument as well as evaluate the range of ECD linearity. The range of concentrations should cover the anticipated concentration of targeted analytes in the unknown samples.

Laboratory method blank A sample that contains every component used to prepare samples except for the analyte(s) of interest via PS–RP–SPE and is taken through the sample preparation process. It is used to evaluate the extent of laboratory contamination for the targeted analytes.

2.8 Quality Control Specifications

Minimum Demonstration of Capability

Before samples are prepared and extracts injected into a GC, the laboratory must demonstrate that the instrumentation is in sound working order and that targeted analytes can be separated and quantitated. GC operating conditions must be established and reproducibility in the GC retention times, $t(R)$, for the targeted analytes must be achieved.

Laboratory Background Contamination

Before samples are prepared and extracts injected into a GC, the laboratory must demonstrate that the sample preparation bench-top area is essentially free of trace OCs and PCBs residues. This is accomplished by preparing method blanks using either distilled, deionized water, or acetonitrile as

reagent blanks and observing essentially peak free GC-ECD chromatograms.

Assessing Targeted and Surrogate Analyte Recovery

Before samples are prepared and extracts injected into a GC, the laboratory must demonstrate that the surrogate and targeted analytes can be recovered by the method to a reasonable degree. EPA Method 508 suggests that an acceptable range in percent recovery can be found to be $\pm 30\%$. Typical values for R range from 65% to 115%.

Assessing Calibration Curve Linearity

Multipoint calibration covering one or more orders of magnitude above the 0.1-ppb instrument detection limit (IDL) for C-GC-ECD based on a 1- μ L injection volume with a 10 : 1 split ratio should satisfy a minimum correlation coefficient of 0.9500. Correlation coefficients are calculated within the Turbochrom (PE-Nelson) software itself or by using a computer program.

Assessing ICV Precision and Accuracy

Precision can be evaluated for the triplicate injections of the ICV following establishment of the multipoint calibration by calculating a confidence limit for the interpolated value for the ICV concentration expressed in ppb.

A relative standard deviation expressed as a percent (% RSD) can also be calculated based on peak areas for the i th component according to

$$\%RSD_i = \frac{s_i}{C_i} \times 100$$

where s_i is the standard deviation in the interpolated concentration for the i th targeted component based on a multipoint calibration and triplicate measurements. %RSD is also called the coefficient of variation.

Both the mean concentration and the confidence limits about the mean concentration should be established for the ICV prior to conducting the sample analysis.

Accuracy can be evaluated based on a determination of the percent relative error according to

$$\% \text{ Error} = \frac{|\bar{C}_i - C_i^{\text{known}}|}{C_i^{\text{known}}} \times 100$$

Statements of precision and accuracy should be established for the ICV, and as long as subsequent measurements of the ICV remain within the established confidence limits, no new calibration curve need be regenerated.

2.9 Reporting Requirements

The client generally dictates what type of reporting format to use. Software for processing raw analytical data into analytical results are usually available at the PC used with the instrument. In the author's laboratory, a sample by sample report can be produced and printed using Turbochrom[®] (PE-Nelson). In this sample report, a tabular format that shows the peaks found, analyte name, analyte concentration and analyte retention time follows:

Peaks No	Analyte Name	Conc. (ppb)	$t(R)$	Peak area
6	Lindane	50.4	1.84	21,033
19	Endrin	153.8	4.01	30,163
24	Methoxychlor	188.1	5.56	17,202

In addition, a summary report in which the file name for the chromatogram is placed in the first column, the name of the sample analyzed is placed in the second column, the concentration, retention time, and peak area for the first chromatographically resolved component is placed in the third column is compiled:

File name	Sample name	Analyte		
		Conc. (ppb)	$t(R)$	Peak area
YA13001	Blank	11.0	3.77	21,245
YA13003	133 ppb ICV	140.0	3.76	298,906
YA13009	#2	1,316.4	3.77	2,832,169

Appendix C: TEQA Applied to Drinking Water

Trace environmental quantitative analysis (TEQA) utilizes various determinative techniques (Chapter 4) in combination with various sample prep techniques (Chapter 3). In this appendix, one specific trace analysis using static headspace sampling automatically coupled to capillary gas chromatography with element specific detection is described. LSQUARES is a computer program developed by the author in BASIC and is used in the quantitative analysis discussed below. The actual program written in GWBASIC is also listed after illustrating its use in TEQA.

1. HOW MUCH THMs ARE IN THE AUTHOR'S DRINKING WATER AND DOES THIS RESULT VARY WITH TEMPERATURE?

To illustrate the external standard mode of instrument calibration and subsequent quantitative analysis, a real analysis of the author's drinking water is sampled at the kitchen faucet and at the refrigerator faucet. Both sampling sites are derived from the same source, municipal drinking water from Meridian Township, Michigan. Both samples were reported to contain all four trihalomethanes. This author was surprised to observe that with the exception of chloroform, the other three THMs were somewhat lower in concentration in tepid water versus refrigerator chilled water! To this

author's surprise, he speculated that the volatile THMs would in some way "escape" to a greater degree from the slightly warmed water, whereas these THMs would tend to remain dissolved to a great extent in the chilled water.

The concentration of each THM is normally distributed in the drinking water sample. There exists a population mean concentration for each THM. We can never know this mean concentration, μ , for each THM from the population; we can, however, by measuring L replicates of the sample, calculate a mean concentration, $\bar{x}$, and a standard deviation, s . We really do not know the standard deviation for the population, σ . We can make L replicate measurements and use t -statistics to define a confidence interval for the population mean using chloroform as our example:

$$\mu_{\text{CHCl}_3} = \bar{x}_{\text{CHCl}_3} \pm \frac{ts}{\sqrt{L}}$$

where we use t for a significance level of $1 - \alpha/2$ and for a number of degrees of freedom, df , where $df = L - 1$. The higher the degree of confidence desired for the mean concentration for chloroform, the larger is the value t required and, hence, the larger the confidence interval.

Calibration data for the four THMs follows:

Conc. THM (ppb)	Peak area ($\mu\text{V s}$)			
	CHCl_3	CHCl_2Br	CHClBr_2	CHBr_3
4	17,235	57,167	30,499	9,749
8	29,148	96,079	52,172	16,319
16	53,881	191,910	105,408	31,905
32	88,720	345,150	176,642	51,009

Upon entering the calibration data above for each of the four THMs, the program titled LSQUARES was used to generate the following statistical parameters. These statistical parameters were used to interpolate the instrument response from the sample and calculate the corresponding concentration along with a confidence interval at the stated level of significance.

Parameter	CHCl ₃	CHCl ₂ Br	CHClBr ₂	CHBr ₃
Slope ($\mu\text{V s/ppb}$)	2,540	10,344	5,234	1,473
y Intercept ($\mu\text{V s}$)	9,143	17,422	12,671	5,150
Correlation coeff.	0.9960	0.9989	0.9957	0.9931
Std. dev. in slope	161.7	347	343	123
Std. dev. in y intercept	2,982	6,402	6,333	2,261
$t(1 - \alpha, df = 3)$	2.353	2.353	2.353	2.353
$y_C (\mu\text{V s})$	19,908	40,528	35,528	13,313
x_C (ppb)	4.2	2.2	4.2	5.5
x_D (ppb)	8.2	4.4	8.4	10.6
Sample				
Concentration in ppb for kitchen faucet at $\sim 30^\circ\text{C}$	24.1	16.3	15.5	3.5
Conf. interval in ppb at 95%	5.2	2.6	5.0	7.0
Concentration in ppb for refrigerator faucet at $\sim 4^\circ\text{C}$	27.1	14.2	12.1	1.9
Conf. interval in ppb at 95%	5.4	2.6	5.0	7.2

2. WHAT IS THE PROGRAM FOR LSQUARES?

The computer program is listed here for those who would like to use it. The mathematical equations that are programmed into GWBASIC could be easily captured and placed into an EXCEL spreadsheet and thus bring this program into the more contemporary Windows[®] environment.

Line No.	Code in GW BASIC
1	INPUT "ENTER Y (ALL CAPS) FOR WEIGHTED LEAST SQUARES, N IF NOT "; U\$
2	IF U\$ = "Y" THEN GOSUB 700 ELSE 5
3	GO TO 323
5	REM THIS PROGRAM ESTABLISHES THE CALIBRATION CURVE FOR X,Y PAIRS OF DATA USING NON-WEIGHTED LEAST SQUARES REGRESSION STATISTICS.
6	REM IT ALSO APPLIES BASIC STATISTICAL CONCEPTS TO FIND THE SLOPE, Y-INTERCEPT AND CORRELATION COEFFICIENT FOR THE LEAST SQUARES REGRESSION.

Line No.	Code in GW BASIC
7	REM IT ALSO FINDS THE STANDARD DEVIATION IN BOTH THE SLOPE AND Y-INTERCEPT AND USES THESE PARAMETERS TO ENABLE AN INTERPOLATION WITH CONFIDENCE INTERVALS SPECIFIED.
8	REM IT ALSO FINDS DETECTION LIMITS USING CONCEPTS FROM Hubaux and Vos, <i>Analytical Chemistry</i> , 1970, 42(8), 849–855 and from Koehn and Zimmerman, "Method Detection Limits, or How Low Can You Really Go: Estimation of Analytical Method Reporting Limits by Statistical Procedures", paper presented at the EPA Conference on Analysis of Pollutants in the Environment Norfolk, VA May 13–14, 1987.
9	DIM X(25), Y(25),A(25),Z(25),C(25)
10	INPUT "HOW MANY DATA POINTS";N
20	FOR I = 1 TO N
25	PRINT
30	INPUT "WHAT IS THE X VALUE"; X(I)
40	IX = IX + X(I)
50	A(I) = X(I)*X(I)
60	HX = HX + A(I)
65	PRINT
66	PRINT
70	INPUT "WHAT IS THE Y VALUE";Y(I)
80	IY = IY + Y(I)
90	Z(I) = X(I)*Y(I)
100	JXY = JXY + Z(I)
110	C(I) = Y(I)*Y(I)
120	KY = KY + C(I)
125	PRINT
130	NEXT I
140	LET L = IX/N
150	LET M = IY/N
160	LET E = IX*IX
170	LET F = IY*IY
180	LET SXX = HX - E/N
190	LET SXY = JXY - (IX*IY)/N
200	LET SYX = KY - (IY*IY)/N
210	LET B = SXY/SXX
220	LET A = M-B*I
230	LET VAR = SYX/(N-2)-(SXY*SXY)/SXX/(N-2)
240	LET R = SXY/SQR(SXX*SYX)
250	LET SDB = SQR(VAR)*1/SQR(SXX)

Line No.	Code in GW BASIC
260	LET SDA = SQR(VAR)*SQR(HX/(N*HX-E))
265	PRINT
270	PRINT "THE SLOPE OF THE REGRESSION LINE IS";B
275	PRINT
280	PRINT "THE Y-INTERCEPT OF THE REGRESSION LINE IS";A
285	PRINT
290	PRINT "THE CORRELATION COEFFICIENT FOR THE REGRESSION IS";R
295	PRINT
300	PRINT "THE STANDARD DEVIATION IN THE SLOPE OF THE REGRESSION LINE IS";SDB
310	PRINT
320	PRINT "THE STANDARD DEVIATION IN THE Y-INTERCEPT IS"; SDA
321	PRINT
322	REM THE CRITICAL LEVEL, YC, (IN RESPONSE UNITS) THE CRITICAL LIMIT, XC (IN CONC OR AMT UNITS), THE INSTRUMENT DETECTION LIMIT, XD, (IN CONC OR AMT UNITS), THE COORDINATE WHICH CORRESPONDS TO XD AND THE REVISED DETECTION LIMIT, XDD, ARE ALL CALCULATED.
323	INPUT "WOULD YOU LIKE TO FIND DETECTION LIMITS FOR WEIGHTED LEAST SQUARES? ENTER Y (ALL CAPS) FOR YES AND N FOR NO";B\$
324	IF B\$ = "Y" THEN 900 ELSE 325
325	INPUT "WOULD YOU LIKE TO FIND DETECTION LIMITS FOR UNWEIGHTED LEAST SQUARES? ENTER Y (ALL CAPS) FOR YES AND N FOR NO";B\$
327	PRINT "YC,XC,XD FOR THIS CALIBRATION CURVE ARE:"YC,XC,XD
328	PRINT
329	PRINT "YD AND XDD FOR THIS CALIBRATION CURVE ARE:"YD,XDD
330	INPUT "WOULD YOU LIKE TO FIND XDD? ENTER Y (ALL CAPS) FOR YES OR N FOR NO";Z\$
331	IF Z\$="Y" THEN GOSUB 640 ELSE 335
332	PRINT "THE INST DET LIMIT ACC TO KOEHN AND ZIMMERMAN, XDD, IS"; XDD
335	INPUT "WOULD YOU LIKE TO INTERPOLATE? ENTER Y FOR YES OR N FOR NO";A\$
340	IF A\$="Y" THEN 345 ELSE 999

TABLE C.1 Input Data for the LSQUARES Calculation of the Decision level and IDL for Trace As via GFAA Using the AANALYST 800

Calibration Curve:		As measurement on AA Furnace				Date	08/11/2000
		Average		Average			
n	10	X*	5.500	Y*	0.017		
STD ID	Conc. (Xi) ug/L	Response (Yi)	(Xi-X*)^2	(Yi-Y*)^2	(Xi-X*)(Yi-Y*)	Y'	(Yi-Y')^2
Std-1	1	0.0032	20.250	0.000194	0.063	0.003209	8.26446E-11
Std-2	2	0.0062	12.250	0.000120	0.038	0.006305	1.09932E-08
Std-3	3	0.0095	6.250	0.000058	0.019	0.009401	9.87916E-09
Std-4	4	0.0126	2.250	0.000021	0.007	0.012496	1.07405E-08
Std-5	5	0.0153	0.250	0.000003	0.001	0.015592	8.53348E-08
Std-6	6	0.0189	0.250	0.000003	0.001	0.018688	4.49954E-08
Std-7	7	0.0222	2.250	0.000026	0.008	0.021784	1.73359E-07
Std-8	8	0.024	6.250	0.000047	0.017	0.024879	7.73334E-07
Std-9	9	0.0287	12.250	0.000134	0.040	0.027975	5.25405E-07
Std-10	10	0.0308	20.250	0.000187	0.061	0.031071	7.33917E-08

Source: PerkinElmer Instruments, Norwalk, CT.

Calibration Curve: As measurement on AA Furnace		Date	08/11/2000
Calibration Curve Regression		Calculation	
Number of Standards (n)	10	X*	5.500
Slope (a)	0.003096	Y*	0.01714
Intercept (b)	0.000113	SSxx	82.50
Linear Correlation Coefficient (R^2)	0.9978	SSyy	0.0008
Calculation of Detection Limit		SSxy	0.26
Confident Level (α)	0.05	Variances	
Critical Level (Lc)	0.336 ug/L	sx	3.028
Detection Limit (LD)	0.651 ug/L	sy	0.009
RUN SOLVER TO GET DETECTION LIMIT !!!		sxy	0.168

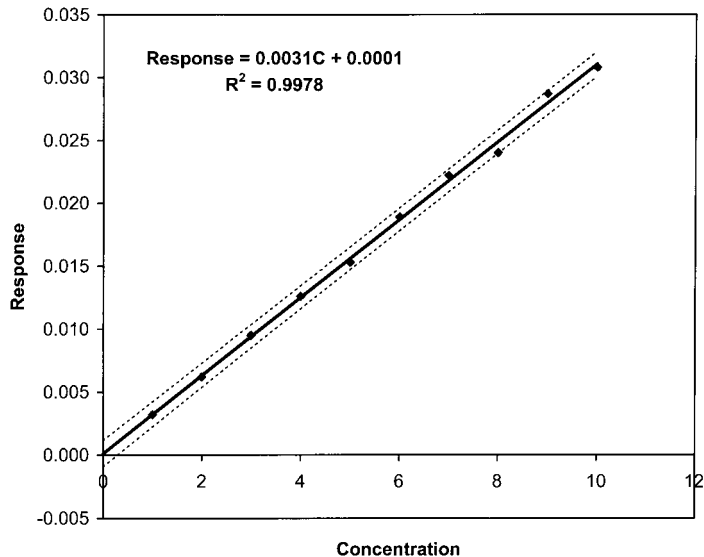


FIGURE C.1 LSQUARES approach to calculating critical level and IDL for trace As via GFAA using the AANALYST 800. (PerkinElmer Instruments, Norwalk, CT.)

Appendix D: Instrument Designs

The field of TEQA is constantly changing as new technologies enable new methods to be developed and implemented. Included in this appendix are a small collection of diagrams and figures that hopefully convey to the reader some of the more recent technological advancements that did not appear in the text.

In Chapter 3, page 131, EPA Method 5030 was introduced. This is the classical purge and trap technique to prepare aqueous and soil samples for determining trace levels of VOCs. Determining trace VOCs has been one of the more controversial topics in TEQA because many of the lower molecular weight VOCs are lost either during sampling in the field or in transfer of the field sample to the purge vessel in the laboratory. EPA Method 5035 has recently been developed to address this loss of VOCs. The AQUATek 70[®] (Tekmar-Dohrmann) is shown in Figure D.1 and is designed to automate the P&T technique as applied to drinking, ground, surface, and waste water samples as defined in EPA Method 5035. The Precept II[®] (Tekmar-Dohrmann) is shown in Figure D.2 and is designed to automate the P&T technique as applied to either aqueous or soil samples. The Precept II interfaced to a Model 3100 Concentrator[®] (Tekmar-Dohrmann), and the transfer line from the Concentrator to a volatiles interface on a Model 6890 GC/5973 N MSD (Agilent Technologies) was used to generate the reconstructed ion chromatogram that appears on the front cover of this book.



FIGURE D.1 Tekmar-Dohrmann's AQUATEk 70 automated purge and trap unit. (Courtesy of Tekmar-Dohrmann, Cincinnati, OH.)



FIGURE D.2 Tekmar-Dohrmann's Precept II automated purge and trap unit. (Courtesy of Tekmar-Dohrmann, Cincinnati, OH.)

The phase diagram that appeared in Figure 3.7, page 108, included arrows that were placed on the diagram to explain the principles underlying accelerated solvent extraction (ASE). ASE is applicable to SVOCs and represents an alternative to Soxhlet extraction (introduced on page 102). A flow chart was also provided on page 110 that depicted the steps in operation of the ASE. The Model 200 ASE[®] (Dionex Corporation) is shown in Figure D.3 and a schematic for how it operates is shown in Figure D.4. Soil, sediment, and other solid samples taken from the environment are placed in the ASE extraction cells, shown located in the upper carousel in Figure D.3, while the extractant is found in the vials located in the lower carousel. The extraction from ASE can then subsequently be cleaned up by conventional sample prep methods.

In Chapter 4 we discussed the inductively coupled plasma–atomic emission spectrometer, (ICP-AES). Some of the more classical instrument



FIGURE D.3 Dionex Corporation's ASE 200. (Courtesy of Dionex Corporation, Salt Lake City, UT.)

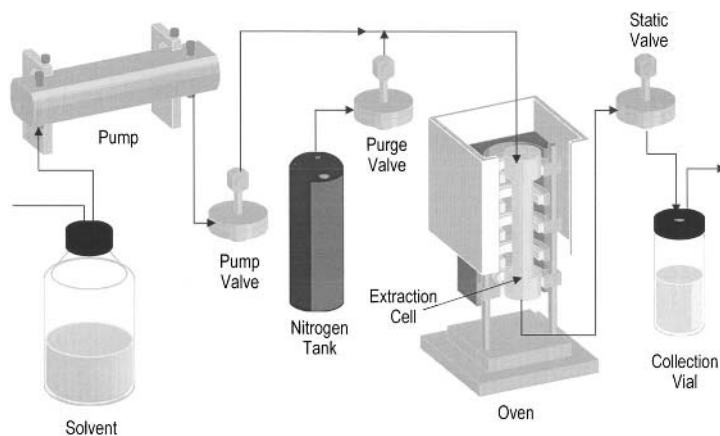


FIGURE D.4 Schematic diagram for the ASE 200. (Courtesy of Dionex Corporation, Salt Lake City, UT.)

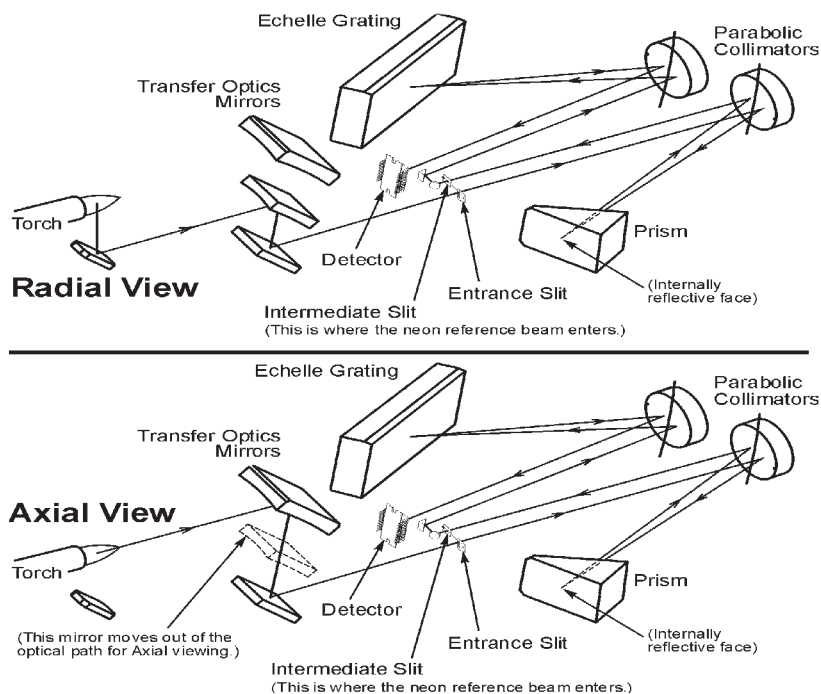


FIGURE D.5 Schematic diagram for the double monochromator design for PerkinElmer Instruments' Optima 2000 DV. (Courtesy of PerkinElmer Instruments, Norwalk, CT.)

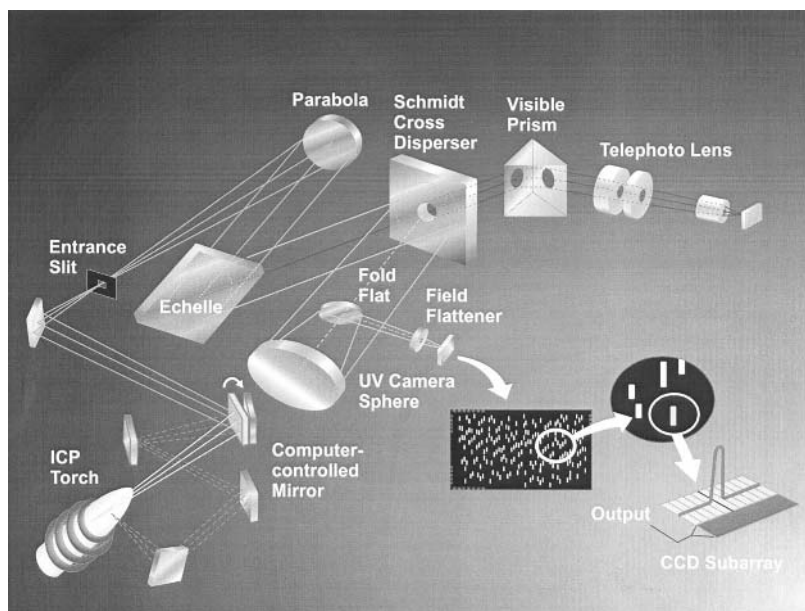


FIGURE D.6 Perspective drawing for the Optima 2000 DV. (Courtesy of PerkinElmer Instruments, Norwalk, CT.)

designs that have contributed to making ICP-AES the determinative technique of choice when the analyst anticipates the need for trace metals analysis were also shown in Chapter 4. The optical layout for a double monochromator design, as is found in the Optima 2000 DV[®] (PerkinElmer Instruments) for the radial and axial positions for the ICP torch, is shown in Figure D.5. A perspective drawing for this instrument is shown in Figure D.6 and gives an idea of how the charged coupled device (CCD) works.

Most companies that manufacture analytical instruments and automated sample prep equipment display drawings and in some cases schematic diagrams on their respective Web Sites. The traditional company glossy brochure or bulletin is rapidly disappearing! It is important to consider instrument specifications when anticipating purchase and to carefully evaluate whether these specifications satisfy the objectives of TEQA.

Appendix E: Useful Internet Links for Environmental Analytical Chemists

The information that is given below appeared in the 1999 Biennial Application Reviews published in *Analytical Chemistry*. These web site listings should help the reader “get connected” with the vast and ever increasing Internet sources:

Clement R, P Yang and C Koester. *Analytical Chemistry* 17:257R–292R, 1999.

Trade journals such as “American Laboratory” and “LC-GC” are beginning to list and annotate existing and new web sites.

METHODS AND TECHNICAL LITERATURE

1. <http://www.epa.gov/epahome/Standards.html>
EPA environment test methods and guidelines
2. <http://www.epa.gov/region01/oarm/links.html>
Links to sources of EPA test methods on the Internet
3. <http://www.epa.gov/ttn/amtic>
EPA’s Ambient Monitoring Technology Information Center: contains details on monitoring methods and related information

4. <http://www.epa.gov/oar/>
Home page for EPA Office of Air and Radiation
5. <http://www.epa.gov/OGWDW/methods/methods.html>
EPA Office of Ground Water and Drinking Water: analytical methods for drinking water
6. <http://www.epa.gov/epaoswer/hazwaste/test/main/htm>
EPA Office of Solid Waste: contains SW-846 on-line test methods for evaluating solid waste physical/chemical methods
7. <http://www.wpi.org/wpi/prodser/chemmethods/chemmeth.asp>
Detailed summaries and comparison of critical parameters of EPA methods; useful for method selection, method modification and data comparability
8. <http://www.sampleprep.duq.edu>
Sample Prep Web: contains information and advice regarding analytical sample preparation, speciated analysis, trace analysis and microwave chemistry
9. <http://www.sigma-aldrich.com>
From menu choose Supelco and then Supelco Technical Library: ~ 100 environmental bulletins and application notes can be downloaded and printed

CERTIFIED REFERENCE MATERIALS (CRMs/SRMs)

1. <http://ois.nist.gov/SRMcatalog/>
Technical and ordering information for National Institute of Standards and Technology (NIST) SRMs
2. <http://www.ems.nrc.ca/ems1.htm>
CRMs of the Canadian National Research Council
3. http://www.pss.aus.net/products/ref_mat.html
U.K. organization that acts as a central supply for CRMs from around the world
4. <http://www.jrc.org/irmm/index.asp>
Institute for Reference Materials and Measurements: promotes European harmonization and standardization in analytical measurements, quality control in preparation of CRMs

ENVIRONMENTAL/ANALYTICAL CONFERENCES AND ORGANIZATIONS

1. <http://www.acs-envchem.duq.edu/>
Home page of the American Chemical Society, Division of Environmental Chemistry

2. <http://www.carleton.ca/~rburk/ea2000/title.htm>
Information concerning the biennial conference EnviroAnalysis; devoted to all aspects of environmental pollution and monitoring
3. <http://www.wpi.org/wtqa/>
Information concerning the (EPA sponsored) Annual Waste Testing and Quality Assurance symposium (WTQA)

LINKS TO RELEVANT ENVIRONMENTAL INFORMATION

1. <http://pwl.netcom.com/~qaa/links.html>
Links to sources of relevant environmental chemistry information
2. <http://www.liv.ac.uk/Chemistry/Links/refanal.html>
Contains many links to analytical chemistry sources of information
3. <http://www.fasor.com/~iso25/>
ISO/IEC Guide: contains extensive links to international accreditation bodies and to standards organizations

Index

- Absorption strength, in atomic absorption, 438
- Accelerated Solvent Extraction (ASE), 108
 - summary of validation study for, 112
- Acetic acid and sodium chloride, 71
- Acetone, 140, 143, 334
- Acetonitrile, methanol, and tetrahydrofuran, 390
- Acid digestions, procedure for determining trace metals, 219
- Activity coefficient for ith solute, 179
- Alkanes, 306, 321
- Alkylbenzene sulfonate, 96
- Alternative sample prep approaches for trace CPH residue analysis, 82
- Alternative soil, sediment sample prep techniques to Soxhlet Liquid-Solid Extraction (S-LSC), 103
- 2-aminoethanols and C-GC-MS(ITD), 371
- Analyte loss, when sample prep involves phase distribution equilibria, 509
- Aniline, and substituted anilines as priority pollutants, 483
- Anionic surfactants via μ LLE, using ion-pairing with methylene blue (**experiment**), 551
- Anions, using IC (**experiment**), 570
- Apparatus:
 - for cold-vapor AA, 246
 - for purge and scrubbing to isolate cyanide from hazardous waste, 248
- Approval of analytical methods, 12
- a-q stability diagram for quadrupole MSDs, 363
- Aqueous solubility:
 - and its relationship to RP-SPE recoveries, 177
 - and methods of estimating, 181
- AR 1242, 29, 210, 212, 213
- Aroclor 1260, 342
- Arsenic (III) and (V), 415
- Assay of a chemical formulation to treat lawns, 503

- Atomic absorption spectroscopy, 433
- Atomic spectroscopy, 412,
- Atomization temperatures in atomic spectroscopy, 419
- Atomization vs. ionization processes, in ICP-AES, 428
- Back extraction, in ultra-micro LLE, 101
- Background correction techniques, in AA, 440
- Barrel vs. disk format for SPE, 160
- Base neutral and acid priority pollutants, 309, 311
- Batch sample sequence, illustrated, 606
- Beer-Bouguer law (*see* Beer-Lambert law)
- Beer-Lambert law, derivation of, 384
- Benzene, 27
- Benzo(a)pyrene, 103
- Benzo(g, h, i)perylene, 155
- BghiP recoveries, comparison of SFE, ASE, S-LSE, U-LSE, and MAE, 156
- Bis (2-ethylhexyl)phthalate, 65, 477
- Bisphenol A, p, p'-DDT, diethylstilbestrol, 3
- Block diagram:
 - for HPLC, 376
 - for ICP-AES, 425
- Boltzmann distribution, in ICP-AES, 427
- Breakthrough volume:
 - in SPE, 170
 - for SPE via RP-HPLC, 187
- Bromate, bromide, chlorite, and chlorate, 403
- BTEX, as VOCs via HS-C-GC-FID (**experiment**), 508
- BTEX GC chromatogram, 294
- BTEXs, 293, 311
- 2-butanone, 140
- Butyl phthalate, interacting with C₁₈ ligates on bonded sorbent, 498
- Ca, Mg, in groundwater via FLAA (**experiment**), 534
- Cadmium oxine complex, 231
- Calibration:
 - of Cr in ICP-AES, 432
 - of Cr using GFAA, 442
 - of a FTIR spectrophotometer for TPHs, 543
 - verification and quantitative analysis for QC/SOP, 607
- Calibration curve (AR 1242) for SPME, 211
- Calibration curve for internal standard mode, 497
- Calibration data, in ES mode for THMs, 614
- Capacity factor, k' :
 - in chromatography, 274
 - and its relationship to the octanol-water partition coefficient, K_{ow}, 186
 - and the required number of theoretical plates, N_{req}, 291
- Capillary electrophoresis (CE), 450
- Carbon dioxide equilibria, equations, and distribution diagram, 570
- Categorization of methods for trace inorganics analysis, 216
- CERCLA, 6
- CFR, 5
- C-GC-ECD (AR 1260) chromatogram, 342
- Challenge to implement an EPA Method, 17
- Chemical potentials of dissolved solutes, 73
- Chloride, nitrite, nitrate, phosphate, and sulfate, 403, 460
- Chlorobenzene, 1, 3 and 1, 4-dichlorobenzene, 353
- Chloroform, carbon tetrachloride, and trichloroethylene, 339, 353
- Chloromethane, 140, 143
- 4-chloro-3-methyl phenol, 65

- Chlorophenoxy acid herbicides (CPHs), 81, 112, 388
- Choosing columns in GC, 302
- Choosing which instrument to use, 414
- Chromate via coprecipitation, 223
- Chromatographic resolution, R_s , 290,
- Chromatography, 268
- Chromium, 54, 222, 431, 442
- chelation with diphenylcarbazone, 223
 - quantitative determination in various soil types, **(experiment)**, 524
 - speciation via EPA Method 7195, 223
- Classification of supercritical fluids, 145
- Clausius-Clapyron equation, and vapor pressure of liquids, 120
- Clean Air Act, 10
- Clean Water Act, 6
- CIVOCs, 257
- Cobalt chloride, visible absorption spectrum, 570
- Coefficient of variation in calculation of percent recovery, 51
- Column liquid phases in GC, 309
- Comparative studies involving different sample prep techniques with soil, 153
- Comparison:
- of CE to HPLC and GC, 451
 - of the ES and SA modes for Pb via GFAA, 533
 - of IDLs among the most common atomic spectroscopic methods, 416
 - of IE vs. IC resins, 405
 - of the most common detectors used in GC, 329
 - of RP-HPLC fixed vs. λ programmed, 484
 - of RRFs, PDECD vs. ECD, 353
 - of two dependent averages, equations for, 514
 - of ultraviolet absorption and fluorescence in HPLC, 397
- [Comparison]
- of UV and IR absorption spectra of organic compounds **(experiment)**, 545
- Concentration of extractant from S-LSE, using K-D or rotary evaporation, 102
- Conceptual framework for MSD, 359
- Conductivity and conductivity detection, 407
- Confidence intervals:
- vs. confidence levels, 52
 - about the least squares regression line, 44
- Configuration for an instructional laboratory, 476
- Consideration of blanks in standard addition mode of instrument calibration, 34
- Contemporary y_Q and x_Q , 54
- Copper, as oxine complex, 92
- Correlation coefficient, defined for the least squares regression line, 38
- Coupling blank and calibration to find limit of quantitation x_{LOQ} , 54
- Craig countercurrent distribution, as basis of separating EG and 1, 2-DCA, 265
- Craig countercurrent extraction, mathematical basis for, 262
- Critical response y_C , 46
- Cu oxinate, between ether and water, 93
- Cyanide trace analysis, sample preparation for, 247
- 2, 4-D, 2, 4, 5-T, and Silvex, 387
- Data acquisition and control using Turbochrom **(experiment)**, 489
- DB-1 vs. DB-5 (AR 1242) chromatogram, 316
- Debye-Huckel equation, 76
- Decachlorobiphenyl, 58, 207, 212
- Decision limit x_C , 48
- Definition:
- of the distribution ratio, D , in LLE, 84

[Definition]

- of equivalent conductance, 408
 - of the fraction extracted and the percent recovery, 91
 - of HETP, 277
 - of hexadecane screening for trace VOCs, 128
 - of the internal standard mode of instrument calibration, 30
 - of a laboratory's QA program, 57
 - of the least squares regression, 34
 - of liquid-liquid extraction (LLE), 71
 - of mass spectral resolution, 362
 - of a solute's aqueous solubility, 178
 - of a solute's chemical potential, 73
 - of specific conductivity, 407
 - of the standard addition mode of instrument calibration, 33
 - of the three modes of instrument calibration, 28
- Definitions, of the major types of column chromatography, 268
- Dependence of k' on the percent organic modifier in the mobile phase in HPLC, 378
- Derivation:
- for acetic acid partitioning in LLE, 85
 - from confidence intervals, 44
 - of equation for the Gibbs Phase Rule, 107
 - of equations for the quadrupole MSD, 360
 - of equations for quantitating VOCs via HS-GC, 117
 - of fraction complexed (Cd-oxine), 234
 - of fundamental equations in chromatography, 276
 - of fundamental relationships metal chelation LLE, 224
 - of the Gibbs Phase Rule, 106
 - of Henry's Law constant, K_H for, 114
 - of the linear least squares regression equations, 34

[Derivation]

- of a more useful equation for metal chelation LLE, 226
 - of quantitative equation for HS-GC, 117
 - of R_s equation in capillary electrophoresis, 455
 - of temperature dependence of specific retention volume, V_g , in GC, 327
 - of the thermodynamic distribution constant K^0 , 74
 - for ultra-micro LLE, 98
- Detection limit x_D , 48
- Detection response y_D , 47
- Detectors:
- in GC, 329
 - for HPLC, 384
- Determinative techniques:
- in TEQA, 255
 - which to choose, 258
- DFTPP, 17
- Diazinon vs. Malathion, 200
- Dibenzo(a, h)anthracene, 65
- 1, 2-dibromopropane, 30
- Dibutyl phthalate, 170
- Dicamba, 387
- 1, 2-dichlorobenzene- d_4 , 143
- 1, 2-dichloroethane, 140
- Difference between reversed-phase columns in HPLC, 382
- Differential flow control in GC, 297
- Differential migration in separations, 259
- Dimensionless z , use of to simplify the Normal distribution, 52
- Dimerization of HOAc in LLE, 88
- 9, 10-dimethyl-1, 2-benzanthracene, 104
- N, N-dimethylformamide, 344
- Dimethyl phthalate, 170, 477
- Dimethyl vs. cyanopropyl siloxanes, for GC capillary column liquid phases, 314
- Diphenylcarbazine, 525
- 2, 4-diphenyl hydrazones of aldehydes, 374

- Disinfectant by-products determined by IC, 403
- Dispersive vs. FTIR instrument designs, 446
- Distribution diagrams for the Cd-oxine complex, 237
- Distribution in Craig countercurrent extraction, 263
- Distribution ratio:
 - for the Cd-oxine complex, 239
 - and its relationship to % recovery, 90
 - and its relationship to K_{IP} , 95
- Distribution Ratio, D , that accounts for secondary equilibria in LLE, 84
- Dodecane (normal), 99
- Domains, to place the major types of chromatographic separations in, 270
- Dominance of GC in TEQA, explained, 270
- Drawing apparatus for fabrication of WCOTs, 312
- Dual mega-bore C-GC-TSD/FPD, 344
- Dual monochromator diagram for ICP-AES, 424
- Echelle grating, monochromator for, 426
- Effect of increasing temperature on static headspace GC, 120
- Effect of longitudinal diffusion in WCOTs, 318
- Electrolytic Conductivity Detector (EICD), 337
- Electromagnetic spectrum, 413
- Electron Capture Detector (ECD), 337
- Elements of Good Laboratory Practice, 26
- Elimination of emulsions in LLE, 130
- Elution, in instrument design for chromatography, 270
- Elution chromatographic approach, to measure SPE breakthroughs, 173
- Emulsions in LLE, 130
- Endocrine-disrupting chemicals, 3
- Endrin, 192
- Energy level diagram, for sodium, 418
- Environmental analytical chemistry:
 - defined, 2
 - literature of, 19
- Environmental catastrophes, 4
- Environmental chemistry, defined, 1
- Environmental testing, as very important, 4
- EPA, establishment of, 2
- EPA Method 413, 14
- EPA Method 200.7, 422
- EPA Method 300.1, 403
- EPA Method 507 using WCOTs, 313
- EPA Method 508, 97
- EPA Method 610, 483
- EPA Method 1664, 540
- EPA Method 3550B, 105
- EPA Method 3562, 151
- EPA Method 4132, 540
- EPA Method 5021, 125
- EPA Method 5030B, 137
- EPA Method 6010B, 527
- EPA Methods 7140 (Ca) and 7450 (Mg), 539
- EPA Method 7190, 527
- EPA Method 7196, 525
- EPA Methods 7470, 7471A, 245
- EPA Method 8270C, as a challenge to implement, 16
- EPA Method 8315, 374
- EPA Methods 8321A and 8325, 384
- EPA Methods, satisfy the regulations, 12
- EPA's approach to finding the limit of quantitation, 53
- EPA's approach to trace VOCs, 130
- EPA's organics protocol for TEQA, 21
- EPA-sponsored study that utilized microwave assisted extraction, 157
- Equation:
 - based on slope/blank, 44

- [Equation]
 - for the Gaussian or Normal distribution, 52
 - for metal chelation LLE, 94
- Equations:
 - for decision limits, x_C , and detection limits x_D , 48
 - for fluorescence quantitation in HPLC, 394
 - for required plates, N_{req} , in GC, 291
 - for slope and intercept for the linear least squares regression line, 38
- Equilibrium constants, for the Cd-oxine complex, 233
- Equilibrium partitioning, of solutes between immiscible phases, 74
- Estimation of the number of theoretical plates, N , in chromatography, 278
- Ethanol, 122
- Ethylene glycol (EG) and 1, 2-dichloroethane (1, 2-DCA), 258
- Ethyl ether, 72
- Evolution of instrumentation for ICP-AES, 427
- Experiment using Chelex 100/Cation Exchange, 401
- Experiments that illustrate principles of TEQA, 471
- External gas pneumatics in GC, 295
- External standard mode of instrument calibration, 28
- Extrapolation to a large number of Craig vessels, 265
- Factors influencing mass transport rate in solid-phase microextraction, SPME, 202
- Factors that influence separation in capillary electrophoresis, CE, 458
- Ferrous ion, Fe^{2+} , quantitative determination of using dual ion-exchange columns, 401
- FID operating characteristics, 334
- FIFRA, 9
- FIAA vs. GFAA, diagrams for, 436
- Flame Ionization Detector (FID), 333
- Flame Photometric Detector (FPD), 344
- Flow chart:
 - for analytical method development using SPE, 165
 - of analytical scheme to distinguish various forms of phosphorous, 562
 - for choosing a WCOT column in GC, 311
 - for implementing EPA Method 8270C, 22
 - for the organization of an environmental testing laboratory, 64
 - for screening decisions to quantitate VOCs, 127
 - for the sequence of steps in operating an ASE instrument, 110
- Flow rates, for the Autosystem® Gas Chromatograph, 520
- Flow-through packed columns, equation, 491
- Fluorescence (FI) detection, in HPLC, 393
- Four-step procedure for conducting SPE, 162
- Fraction of undissociated HOAc, α , 88
- Fundamental basis of liquid-liquid, LLE, 71
- Gas chromatography (GC), 293
 - description of Autosystem® Gas Chromatograph, 519
 - evaluating parameters that influence performance (**experiment**), 518
- Gaseous SO_2 , reaction with H_2O , 114
- GC chromatogram, of BTEX components from water contaminated with gasoline, 129
- GC chromatogram (AR 1242) from, 208
- GC-FTIR, 441

- GC-MS, using a quadrupole mass spectrometer, 480
- Gel Permeation Chromatographic Cleanup, 11
- Getting started doing supercritical fluid extraction (SFE), 144
- GFAA:
 - description of dry, ash, and atomize steps, 529
 - sequence of operational procedures, 532
- Gibbs Free-Energy, 73
- Gibbs Phase Rule, 107
- Glass vessels for Craig countercurrent extraction, description of, 261
- Glossary, in TEQA, 585
- Golay equation, for WCOTs or other open tubular GC columns, 315
- Good Laboratory Practice (GLP), 26
- Graphical interpretation:
 - of a hypothetical plot of Gibbs free energy vs. mole fraction, 77
 - of the linear least squares regression fit with statistical treatment, 47
- Graphical view of a phase diagram for any chemical substance, 108
- Groundwater ion chromatogram, 411
- Headspace sampling using a manual technique, 511
- Hexadecane, 335
- Hexadecane LLE to isolate, recover and quantitate BTEX, 515,
- Hexadecane screening, for trace VOCs, 128
- Hexane (normal), 122, 334
- High Performance Liquid Chromatography (HPLC), 393
 - as a complement to GC, 372
 - depicted in terms of five zones, 491
 - historical development of, 377
 - introduction to (**experiment**), 489
 - universal nature of, 272
- Historical development:
 - of AA, 433
 - [Historical development]
 - of CE, 451
 - of HPLC, 377
 - of IC, 401
 - of ICP-AES, 422
 - of SPE, 160
 - of Student's *t*-statistics, 41
 - Historical developments, in atomic spectroscopy, 417
 - Historical significance, of LLE, 71
 - Holding times, in trace VOCs analysis, 137
- Inductively-coupled plasma atomic emission spectroscopy (ICP-AES), 422
 - basis for quantitation, 431
 - historical development of, 422
- IDL, illustrated in QC/SOP, 608
- Influence:
 - of chemical nature of the elution solvent in SPE, 198
 - of film thickness for WCOT, 315
 - of hydroxide ion on metal chelation, 230
 - of pH in migration time in CE, 463
- Infrared absorption spectroscopy, in TEQA, 441
- Infrared spectroscopy:
 - importance of to TEQA, 441
 - application of Beer's law to, 447
- Initial Calibration Verification Standards, 26
- Injection techniques in GC, 298
- Inorganic trace analysis and categorizing methods, 216
- Instrument calibration, importance of, 27
- Instrument Detection Limit (IDL or x_D), as important in GLP, 26
- Instrument Detection Limits (IDLs), 43
- Inter versus intramolecular interactions, 176
- Interferences in AA, 443

- Internal standard (IS) mode:
 - of GC-ECD calibration for OCs, 496
 - of instrument calibration, 30
- Interpolation of and uncertainty in the least squares regression line, 39
- Interpretation:
 - of chromatographic resolution, R_s , 290
 - of the Gibbs Phase Rule, 107
 - of the phase diagram for a chemical substance, 107
- Ion chromatograph, schematic of classic instrument, 571
- Ion Chromatography (IC), 401
- Ion-Exchange (IE), in TEQA, 399
- Ion-exchange chemistry for suppressed anion IC, 407
- Ion-limiting equivalent conductivity, 410
- Ion pair with anionic surfactants, 96
- Ion pairing in LLE, 94
- Ion Trap MSD, 366
- IPA (2-propanol), 76
- IR absorption, discovery of, 444
- Iron, determination of trace levels of iron in groundwater (**experiment**), 555
- Isolation and recovery of TPHs by LLE, 97
- k' and the required number of theoretical plates, N_{req} in GC, 292
- Kinetics:
 - of purging VOCs, 138
 - in ultra-micro LLE, 100
- Kovat's retention index in, 306
- K_{ow} for various substituted phenols, 178
- K_{ow} values, where to find, 186
- Kuderna-Danish evaporative concentrator, 483
- Lab X vs. Lab Y, 65
- Laboratory specifications for Quality Control, 59
- Langmuir isotherm, for adsorption of solutes onto solid surfaces, 168
- Lead in drinking water via GFAA (**experiment**), 528
- Least squares linear regression of the calibration curve, 34
- Limit of quantitation, x_{LOQ} , 53
- Limitations of range of organic compounds amenable to GC, 518
- Limitations to metal chelate LLE in TEQA, 94
- Lindane, 192, 195
 - recovered from myometrium cells, 195
- Lindane, endrin, and methoxychlor, 498
- Line broadening, in ICP-AES, 431
- Linear vs. volumetric flow rate in chromatography, 284
- Liquid phases and McReynolds constants in GC, 310
- Liquid-Liquid Extraction (LLE), 70
- LLE:
 - as a cleanup technique, 80
 - as a cleanup technique in sample preparation, 81
- Log K_{ow} versus log S_{aq} for pollutants, 183
- Longitudinal diffusion in chromatography, 281
- Lowering detection limits for determinative instrumental techniques, 257
- LSQUARES, listing of a computer program written in BASIC, 615,
- L'vov platforms for GFAA in, 437
- Make-up gas in GC, 519
- Mass selective detector (MSD), 356
- Mass spectrometers, importance of, 356
- Mathieu equation:
 - for ITD, 368
 - for quadrupole MSD, 362

- Matrix modifiers, for graphite furnace atomic absorption (GFAA), 220
- Maximum contaminant levels (MCLs), 4
- MBAS active substances, 96
- McReynolds constants, 307
- McReynolds probes, 308
- MDL:
- vs. IDL, 50
 - illustrated in QC/SOP, 609
- Megabore capillary GC column, 13
- Mercury, sample prep in the determination of, 245
- Metal chelation:
- with ADPC, 223
 - in LLE, 91
- Metal chelation LLE, 224
- Metal chelation SPE, 231
- Metal dithizonates, 228
- Metal speciation, hyphenated techniques, 463
- Metals, toxicology of, 218
- Method Detection Limits (MDLs), 50
- Methods of Air Sampling, introduced, 19
- Methoxychlor, 192
- 4-methylacetophenone, 99
- N-methylcarbamates, 490
- Methyl ethyl ketone, 122
- Methyl tert-butyl ether, 313
- Methylene blue, 96
- as a cationic ion-pairing agent in IP-LLE, 95
- Methylene chloride, 353, 370
- Micro-LLE, as distinguished from LLE, 97
- Micro-LLE or mini-LLE, 97
- Microsyringe design for SPME, 201
- Microwave-Assisted Extraction (MAE), 156
- Mid-infrared absorption spectroscopy, practical considerations, 548
- Migration times in CE from +30 to -30 kV, 459
- Minimum specifications, for sampling in TEQA, 67
- Mole fraction of saturated solute in water, x_w^{sat} , 179
- Molecular structure of a generalized organophosphorus pesticide, 189
- Molecular structures for eleven PAHs, 488
- N/P pesticides/herbicides, 315
- Nernst solute distribution between immiscible phases, 74, 79
- NIOSH Methods, introduced, 19
- Nitrogen Chemiluminescence Detector, 355
- Nomenclature in HPLC, 375
- Normal distribution, 49
- in Craig countercurrent extraction, 266
- Normal vs. "reversed"-phase HPLC, 377
- NPDES, 8, 483
- Numerically-identified EPA Methods, 14
- Octanol-water partition coefficient, K_{ow} , and molar volume, 178
- Octanol-Water Partition Coefficients (K_{ow}), 177
- Oil and Grease and TPHs in wastewater (**experiment**), 540
- Operation of an ion chromatograph, 572
- Organic modifiers, and Gibbs Phase Rule, 147
- Organics protocol, physical/chemical basis of, 20
- Organization of an Environmental Testing Laboratory, 63
- Organochlorine pesticides (OCs), 112, 304, 311
- isolated from soil and water by RP-SPE, 192
 - μ LLE and RP-SPE (**experiment**), 495

- Organophosphorous pesticides (OPs),
112, 189, 191, 344
isolated and recovered from spiked
water by RP-SPE, 190
- Origin of electro-osmosis in CE, 454
- Oscillation strengths in AA, 439
- Oxine (8-hydroxyquinoline), 92, 232
- P' for selected solvents, 175
- PAHs, 112
in contaminated soil using RP-
HPLC-PDA (**experiment**), 483
extracted from urban dust by, 111
sensitivity and linearity data, UV
absorption, 485
- Partition coefficient for SPME, 203
- Partition constant:
for static headspace VOCs, 119
vs. temperature for static headspace
VOCs, 121
- Partition Ratio, K_D , in LLE, 79
- PCB congener recoveries S-LSC vs.
SFE, 150
- PCBs, congeners, 69, 150, 207
- Percent recoveries C_8 vs. C_{18} in SPE,
191
- Percent recovery:
for the i th analyte, 50
and its relationship to successive
LLEs, 91
of OCs/PCBs, equation for in QC/
SOP, 606
of TPHs from wastewater, 542
- Perchloroethylene (tetrachloroethene),
5, 119, 122
- Performance-based methods (PBM), 13
- Performing least squares regression on
the raw calibration data, 26
- pH measurement, of snow and
contaminated soil, 579
- $pH_{1/2}$, for various metal dithizonates, 228
- Phase diagram, for a chemical
substance, 108
- Phase diagrams:
as a means to explain sample prep,
106
[Phase diagrams]
used to explain sample prep
techniques, 106
- Phase ratio, β , for WCOTs, importance
of, 320
- Phenols:
found after LLE using methylene
chloride, 372
 K_{ow} for, 178
- Phosphorous, determination in
eutrophicated surface water, 561
- Photodiode array (PDA) HPLC-UV
data, 478
- Photo-ionization Detector (PID) in GC
335
- Phthalate ester, breakthroughs in, 172
- Phthalate esters:
low vs. high molecular weight, RP
vs. NP HPLC, 490
using HPLC and GC-MS
(**experiment**), 477
- Phthalic acids, as an HPLC reference
standard, 492
- Physical properties:
hexadecane vs. n-hexane, 128
of SF fluids in, 146
- Physico-chemical basis for solute
equilibration into the headspace,
118
- Planning for taking a "representative
sample", 66
- Plot:
of a liquid's vapor pressure vs. its
temperature, 121
of n vs. sorption time for, 211
of log D vs. pH at differ C_{Hox} , 241
of log D vs. pH, 230
- Plot $1/D$ vs. $[H^+]$ for HOAc, 87
- Polarity of selected organic solvents,
 P' , 175
- Pollution, catastrophic vs. subtle
definitions of, 4
- Polychlorinated Biphenyls (PCBs), 311
- Polycyclic aromatic hydrocarbons
(PAHs), 397

- Practical calculation of HETP, 279
- Preconcentration techniques for trace metals in TEQA, 222
- Predicting:
 K_{ow} from molecular structures, 183
 N and ΔP in HPLC, 383
- Predictions:
 experimental vs. theoretical, 244
 of RP-SPE breakthroughs, 186
- Preparation of reference stock standards in IC, 574
- Primary Drinking Water Monitoring Requirements, 7
- Principal instrumentation for determinative techniques in TEQA, 257
- Principles:
 of C-H stretching, 445
 of the operation of mass spectrometers in TEQA 357, 366
 of purge and trap, 132
- Procedure:
 for alkaline digestion to determine Cr, 526
 to conduct μ LLE, 517
 to determine based on 40 CFR, 43
 to extract PAHs from contaminated soil, 487
 for μ LLE for MBAS, 554
 to obtain FTIR(transmission) spectra, 550
 for visible spectrophotometric determination of Cr, 526
- Programmed temperature gas chromatography (PTGC), 323
- Propagation of error in the calculation of the percent recovery of analyte *i*, 51
- Pulsed Discharge Detector for GC, 346
- Pulsed Discharge Electron Capture Detector for GC, 351
- Purge and trap devices in GC, description of, 135
- Purge and trap techniques, 132
- Purge vessel used in determining VOCs, 134
- Pyrrolidine dithiocarbamate (APDC), 223
- QC reference standards, illustrated in QC/SOP, 609
- QC specifications, defined in QC/SOP, 610
- Quality Assurance, as distinguished from QC, 57
- Quality control (QC)
 reference standards, 58
 specifications that a lab must meet, 59
- Quality control (QC) / Standard Operating Procedure (SOP) plan, illustrated, 604
- Quantitative analysis:
 of author's drinking water for THMs, results for, 615
 using single point external standard R_F , 29
 using single point internal standard RR_F , 31
- RCRA, 6
- Recovery study of SVOCs, 105
- Regulations for environmental monitoring, 5
- Regulatory Methods in TEQA, how classified, 14
- Relative response factor, 31
- Relative standard deviation, in the calculation of an analyte's percent recovery, 51
- Reporting format used in TEQA, 196
- Reporting requirements in QC/SOP, 612
- Requirements for trap sorbents in P&T GC, 133
- Residual for the *i*th calibration point, 34
- Resolution, R_s in chromatography, 287

- Response factor, for external standard mode of instrument calibration, 29
- Restrictor in SFE, 149
- Reversed-Phase SPE (RP-SPE), 159
- percent recoveries, 192
- from soils, 192
- RRFs, for various halogenated HCs, 340
- Safe Drinking Water Act, 6
- Saha equation in ICP-AES, 430
- Salting out and ionic strength LLE, 75
- Sample overload, in GC, 302
- Sample prep for soils, sludges, sediments, 221
- Sample preparation techniques, in TEQA, 69
- importance of, 69
- Sampling techniques, in TEQA, 66
- SARA, 10
- Schedule, for specific laboratory experiments, 472
- Schematic:
- of ejecting m/e ions out of an ITD, 370
 - of a gas chromatograph, 293
 - of instrumental configuration for IC, 403
 - of a mass spectrometer, 367
 - of a P&T GC techniques, 136
 - of a reservoir and barrel for SPE, 161
- Secondary drinking water monitoring requirements, 10
- Secondary equilibria:
- involved for the Cd-oxine complex, 231
 - in LLE, 83
- Selecting the most suitable extracting solvent, 515
- Selection of a suitable IS for determining OCs, 499
- Selectivity, α , and chromatographic resolution, 290
- Sensitivity in HS-GC, 117
- Separation of C₁ to C₆ aldehydic dinitrophenylhydrazones, 373
- Separation of EG from 1, 2-DCA, 259
- SFE-SFC, 104
- Single vs. double beam instruments for AA, 435
- Sodium, energy level diagram for, 418
- Sodium bicarbonate/sodium carbonate eluent, used in suppressed anion IC, 410
- Solid-Phase Extraction (SPE), 158
- as a frontal chromatographic technique, 169
- Solid-Phase Microextraction (SPME), 200
- non-equilibrium considerations in, 204
- Solute activities, 75
- Solute aqueous solubility, 178
- Solute polarity and sensitivity in HS-GC, 123
- Solute trapping techniques in SFE, 150
- Solvent polarity based on a test solute probe, 175
- Solvents:
- and physico-chemical properties for, 389
 - UV cutoffs, 548
- Source, optics and detector designs for, 420
- Sources of ECD contamination, 339
- Soxhlet extraction (S-LSE), 102
- SPE:
- as evolved from HPLC, 158
 - isolation and recovery of TPHs from wastewater, 544
 - literature of SPE, 164
 - typical extraction sequence used to isolate OCs, 496
 - use of a second cartridge to remove moisture, 501
 - and van der Waals forces depicted, 503
- Split ratio, and how to set it in GC, 519

- Split vs. splitless injection in GC, 299
- SPME (*see* Solid-Phase Microextraction)
- Standard addition mode of instrument calibration, 33
- Standard deviation in the vertical residuals, 39
- Standard Methods*, introduced, 19
- Standards for conducting QC in the lab, 58
- Standards preparation, practical considerations, 553
- Static headspace gas chromatographic (HS-GC) analysis, 116
- reasons for lack of EPA acceptance of, 124
- Static vs. dynamic vessels for, 149
- Student's *t*-statistics, 41
- Sulfur Chemiluminescence Detector, 354
- Sulfur dioxide:
- as a model to explain static HS sampling, 114
- sulfurous acid, 114
- Supercritical Fluid Extraction (SFE), 144
- getting started doing, 144
- Suppressed IC explained, 571
- Suppressed vs. non-suppressed IC, 402
- SVOCs: 112
- found in environmental aqueous samples, 518
- SW-846 Methods in TEQA, 11
- Synthetic chemical production, 2
- Systematic error in calibration, 27
- TCE, calibrated by an external standard mode, 32
- TCMX (tetrachloro-*m*-xylene), 58, 167, 185, 207, 212
- TCTFE (1, 1, 2-trichlorotrifluoroethane), 14, 97
- Techniques:
- to eliminate emulsions in LLE, 130
- used to measure dead time, t_0 in GC, 285
- Temperature and selectivity, α , and R_s for WCOTs, 320
- Tenax (2, 6-diphenylene oxide polymer), 133
- TEQA:
- defined, 1
- establishing a calibration curve to conduct, 26
- experiments that illustrate principles of, 471
- glossary, 585
- importance of sample preparation to, 69
- Ion-Exchange (IE) in, 399
- reporting format used, 196
- sample preparation techniques in, 69
- Terms, used to interpret chromatogram in, 276
- 1, 1, 1, 2-tetrachloroethane, 140, 143
- Tetrachloro-*m*-xylene and decachlorobiphenyl, 318
- Thermal Conductivity Detector, 332
- Thermionic Specific Detector in GC, 343
- Thermodynamic:
- explanation of, 73
- view of static headspace, 113
- Thermodynamic Distribution Constant, K^0 , 79
- THMs (*see* Trihalomethanes)
- Three basic functions of MSDs, 356
- Toluene, 122
- Total Petroleum Hydrocarbons (TPHs), 111
- as distinguished for Oil and Grease, 540
- as extracted from soils by ASE, 111
- Trajectory of ions through quadrupole, 364
- Trap used in P&T GC, 134
- 1, 2, 4-trichlorobenzene, 140
- Trichlorobenzene, used to evaluate column overload in GC, 522
- 2, 4, 6-trichlorobiphenyl vs. 4-hydroxy-2, 4, 6, 199

- Trichloroethylene (trichloroethene), 31, 116
- Trifluoroacetic acid, 80
- Trifluralin, in chemically treated lawn soil (**experiment**), 502
- Trihalomethanes (THMs), 28, 311
- determination of, in chilled vs. tepid drinking water, 613
- found in drinking water, 512
- isolated via LLE, 113
- Triphenyl and tricresyl phosphates, 153
- TSCA, 10
- Type I and II errors in estimating x_C and x_D , 56
- Ultrasonic liquid-solid extraction (ULSC), 104
- Ultraviolet absorption spectra, 387
- for CPHs, 388
- Ultraviolet and fluorescence chromatograms, 396
- Ultraviolet molecular absorption and quantized energy levels, 546
- Universal nature of HPLC, 272
- Useful mathematical relationships for chromatography, 273
- Variation in K_{HS}^{VOC} , 122
- Vinyl chloride (chloroethene), 40
- Visible spectrophotometry (**experiment**), 565
- Visual depiction of chromatography, 273
- VOC half-lives, 143
- VOCs via LLE, HS, and P & T sample preparation techniques, 113
- Waters 2487 Uv-vis detector for HPLC, 390
- Waters 474 fluorescence detector for HPLC, 394
- Wavelength most common for HPLC-UV, 483
- Wavenumber notation in infrared spectroscopy, 417
- WCOT GC columns, 311
- Working calibration standards:
- using ES for BTEX, 510
- using ES in IC, 575
- using ES to quantitate OCs/PCBs, 607
- using IS for trifluralin, 506
- using SA for Pb, 533
- X_{LOQ} for Cr, 55
- Zone dispersion vs. zone resolution, 260